Attorney Docket No. 627-31 CT PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: William S. Caldwell et al. Group Art Unit: 1624
Serial No.: 09/522,117 Examiner: V. Balasubramanian
Filed: March 9, 2000
For: COMPOUNDS CAPABLE OF ACTIVATING CHOLINERGIC RECEPTORS

Commissioner for Patents
Washington, DC 20231

**DECLARATION UNDER 37 CFR §1.132**

I, William S. Caldwell, do hereby declare and say as follows:

1. I am a co-inventor on the above-referenced patent application and am familiar with the contents thereof. I have reviewed the final Official Action mailed May 21, 2001 and am familiar with the contents thereof. I have also reviewed U.S. Patent No. 5,861,423 to Caldwell et al. (hereinafter Caldwell), which is cited in the final Office Action.

2. I received my B.S. in chemistry from The University of the South in 1976. I received my Ph.D. in chemistry from The University of Wisconsin in 1986, where I concentrated on Organic Chemistry and Enzymology. From 1985 to 1987 I was a post-doctoral researcher in the laboratory of Dr. Mark Jaffe, a professor at Wake Forest University, where I conducted research in Plant Physiology (chemical correlates of floral induction). From 1987 to 1998 I was a researcher and research manager at R.J. Reynolds Tobacco where my technical duties included research in physical organic chemistry, mechanistic toxicology, xenobiotic metabolism, pharmacology and medicinal chemistry. I have published over 80 papers, book chapters and abstracts and am an inventor on over 40 pharmaceutical-related patents and patent applications. Since 1999, I have been Senior Manager, Director and Vice President for Drug Discovery at Targacept, Inc., where I am responsible for directing all activities in the Drug Discovery Department including Molecular Design, Medicinal Chemistry, Analytical Chemistry/QA, Pharmaceutics and Process R&D. I also hold adjunct faculty appointments in the Chemistry Department of Wake Forest University and in the Physiology/Pharmacology Department at Wake Forest University School of Medicine.

4. I am also a co-inventor on the Caldwell reference that is cited in the final Office Action. This reference describes pharmaceutical compounds having a pyridine ring coupled to an amino group by an unsubstituted alkylene bridging moiety. I was involved in the development and evaluation of the compounds of Caldwell. While these compounds originally looked promising in *in vitro* tests, such as the tests described in the Examples of Caldwell, we found through *in vivo* testing that compounds having unsubstituted alkylene bridging moieties such as those proposed in Caldwell were readily metabolized, and attained only relatively low circulating plasma levels in test animals. As a result, such compounds did not possess an optimum pharmacokinetic profile.

We were interested in discovering compounds that (1) possessed good binding and functional characteristics (*e.g.*, characteristics comparable to those possessed by compounds having unsubstituted alkylene bridging moieties, such as those of Caldwell) and (2) possessed good pharmacokinetic profiles (*e.g.*, were not readily metabolized in the body).

5. In general, the binding characteristics of a particular compound may be evaluated by determining an inhibition constant (Ki value) for the compound. Ki values are reported in units of concentration (nM). Ki values may be calculated from $IC_{50}$ values as described in the present specification at pages 29-31. $IC_{50}$ values are estimated as the concentration of compound that inhibited 50 percent of specific L-[$^3$H]nicotine binding. Thus, better binding is evidenced by a lower Ki value, which indicates that a lower concentration of compound was needed to inhibit specific L-[$^3$H]nicotine binding. In general, utilizing the disclosed method of determining Ki values results in Ki values that are accurate to within approximately a factor of 2. I understand that during the prosecution of this application, our attorneys may have indicated that higher Ki values are preferable and/or that Ki values possess greater accuracy than that just described. In order to clarify any possible misunderstanding, these assertions are not technically correct. It is my understanding that they were not made with an intent to deceive the Patent Office, however.

In general, pharmacokinetic profile characteristics of a particular compound may be evaluated by administering the compound to a subject and determining the plasma levels of the compound over a given time. Two useful measures of a pharmacokinetic profile are

$Cp_{max}$, which is an estimate of the maximum plasma level of the compound, and AUC (area under the plot of plasma concentration of drug against time after drug administration), which is useful in estimating the bioavailability of the compound. For more preferred pharmacokinetic profiles, the $Cp_{max}$ and AUC will generally be higher.

6. In an effort to provide a compound that possessed both good binding/functional characteristics and good pharmacokinetic profiles, we initially attempted to vary the substituent at the 5-position of the pyridine ring of the N-methyl-4-(3-pyridinyl)-3-buten-1 amine compound described in Caldwell. As illustrated by the data in Table 1 at Appendix A, varying the substituent at the 5-position of the pyridine ring resulted in compounds that retained binding at $\alpha4\beta2$ receptor as evidenced by the Ki value. However, such variations at the 5-position did not solve the metabolism problem. All compounds in Table 1 exhibit relatively low circulating levels as evidenced by the $Cp_{max}$ and AUC values. These results, and the isolation of metabolites, indicated to us that the problem might be monoamine oxidase activity at the secondary amine side chain.

7. We then tried various substitutions at other positions on the N-methyl-4-(3-pyridinyl)-3-buten-1 amine compound described by Caldwell. In one compound, we added a single methyl group at the 2-position of the pyridine ring. In another compound, we added a single methyl group at the 6-position of the pyridine ring. In still another compound, we added a single methyl group to the amino group, thus forming a tertiary amine. In yet another compound, we changed the N-methyl group to an N-isopropyl group. As illustrated by the Ki values for the structures shown in Table 2 at Appendix B, we found that these substitutions resulted in dramatically reduced binding characteristics.

8. After the various trials detailed in paragraphs 6 and 7 above, we were inspired to consider another pharmacological field, that of β-phenethylamine and amphetamine chemistry. We reviewed various references describing the pharmacokinetics of phenethylamine and amphetamine chemistry including Shannon, et al., "Physiologic Effects and Plasma Kinetics of β-Phenylethylamine and Its N-Methyl Homolog in the Dog", *J.*

*Pharmacol. Exp. Ther.*, **223**(1): 190-196 (1982); Baggot, et al., "Comparative Study of the Pharmacokinetics of Amphetamine", *Res. Vet. Sci.*, **14**(2): 207-215 (1973); Baggot, et al., "Pharmacokinetic Study of Amphetamine Elimination in Dogs and Swine", *Biochem. Pharmacol.*, **21**(14): 1967-1976 (1972); and Edgar, et al., "Pharmacokinetics of Methamphetamine Self-administered to Human Subjects by Smoking S-(+)-Methamphetamine Hydrochloride", **21**(4): 717-723 (1993). In our review of the literature, we found that installation of a methyl group α to (*i.e.*, on the carbon attached to) the amine nitrogen was known to retard the action of monoamine oxidase for β-phenethylamine and amphetamine compounds, and thus prolong the plasma half-life of these compounds (*see*, Table 3 at Appendix C).

Although Applicants do not wish to be bound by a single theory, the probable explanation of this effect is that the α-methyl group creates a degree of steric hindrance, relative to the unsubstituted material, which hinders binding at the oxidase active site.

9. We believed that the addition of a methyl, or other alkyl group, alpha to the amino group in the compounds of Caldwell could result in a $-CH(CH_3)NH(CH_3)$ amino structure similar to the amino structure of α-methyl versions of β-phenethylamines (*i.e.*, amphetamines). If the poor metabolic characteristics of the compounds of Caldwell were, in fact, due to monoamine activity at the secondary amine side chain, we hoped that employing a structure similar to the amino structure of α-methyl versions of β-phenethylamines might result in the desired improvement in metabolic characteristics.

However, we realized that various structural differences exist between metanicotine compounds, such as those described in Caldwell, and amphetamine compounds. For example, metanicotines have a pyridinyl ring while amphetamines have a phenyl ring. Also, metanicotines have an alkenyl bridging moiety while amphetamines have an alkyl bridging moiety. Given these structural differences, we were not certain that employing an amino structure on the metanicotine compound similar to the amino structure of α-methyl versions of β-phenethylamines would decrease the monoamine activity at the secondary amine side chain and result in the desired improvement in metabolic characteristics.

Even if such an α-methyl amino structure did result in decreased monoamine activity at the secondary amine side chain of the metanicotine compound, we were concerned that this α-methyl structure could result in an undesirable and/or unacceptable reduction in binding at the α4β2 receptor. As described above in paragraph 8, we believed that the improved metabolic characteristics observed in the amphetamine art might be due to the α-methyl group creating a degree of steric hindrance, relative to the unsubstituted material, which may hinder binding at the oxidase active site. We were concerned that the degree of steric hindrance that resulted in hindered binding at the oxidase active sight providing improved metabolic characteristics might also hinder binding at the α4β2 receptor resulting in an undesirable and/or unacceptable reduction in activity at the α4β2 receptor.

These concerns were heightened in view of our experience with the methyl substitutions described above in paragraph 7. Recall, for example, that addition of a methyl group to the amino group had resulted in a drastic decrease in binding at the α4β2 receptor.

Moreover, as summarized in Table 4 at Appendix D, our own experience with the effect of substituents on the binding of (S)-(-)-nicotine at the α4β2 receptor indicated that addition of a methyl group alpha to the amino group may result in an unacceptable decrease in binding. For example, we had previously determined that substituting a methyl group alpha to the cationic site in (S)-(-)-nicotine (Compound 5 in Table 4) resulted in a Ki value of 6400 nM, which was drastically worse than the Ki value of 2 for (S)-(-)-nicotine (Compound 1 in Table 4).

Furthermore, the experience of other researchers in the field indicated that the addition of a methyl group alpha to the cationic site in (S)-(-)-nicotine could result in decreased binding at the α4β2 receptor. For example, Lin, et al., "Synthesis and Evaluation of Nicotine Analogs as Neuronal Nicotine Acetylcholine Receptor Ligands", *J. Med. Chem.*, **37**: 3542-3553 (1994), provided at Appendix E, gives Ki values for α-methyl substituted (S)-(-)-nicotine that are a factor of about 30 to over 1000 higher than the Ki value for (S)-(-)-nicotine (*see*, Lin, et al., page 3545, table 1, compounds 35 and 36 compared with compound (S)-nicotine). Based on our own experience and the experience of other researchers in the field, we thought it likely that the same steric factors responsible for decreased binding at the oxidase might result in decreased binding to the nicotinic receptor.

10. We were also inspired to consider the effect of the stereochemistry of the α-methyl group. By reviewing the literature, we discovered that for various other classes of nicotinic compounds (including nicotine itself), stereochemistry at the site of substitution may be an important determinant of nicotinic binding. For example, the Lin article, discussed above in paragraph 9 and provided at Appendix E, reported that the (S)-nicotine compound having an α-methyl group of alpha stereochemistry (*cis* to the pyridine ring) had a Ki value of about 1205 nM, while the (S)-nicotine compound having an α-methyl group of beta stereochemistry (*trans* to the pyridine ring) had a Ki value of about 35. Thus, while the Lin article had unfavorably showed that the α-methyl group may inhibit binding at the α4β2 receptor, it appeared to show that perhaps one of the two enantiomers of the α-methyl compounds according to embodiments of the present invention might possess binding characteristics that were better than the binding characteristics of the other enantiomer. Such a conclusion was by no means a certainty, however, given the difference in structure between the (S)-nicotine compound and the metanicotine compounds.

Our review of the literature regarding the stereochemical effects of substituents on acyclic amines such as metanicotine revealed WO 96/08468 to Falch, provided at Appendix I, which may be the most relevant art in this area. Falch reports $IC_{50}$ values for two sets of enantiomers having a methyl group alpha to the amino moiety of an acyclic cationic side chain. As described above in paragraph 5, $IC_{50}$ values are estimated as the concentration of compound that inhibited 50 percent of specific L-[$^3$H]nicotine binding. Thus, better binding is evidenced by a lower $IC_{50}$ value, which indicates that a lower concentration of compound was needed to inhibit specific L-[$^3$H]nicotine binding. As shown in Table 2 on Page 16 of Falch, compound (S)-8 has an $IC_{50}$ value of 0.064 μM while compound (R)-8 has an $IC_{50}$ value of 0.003 μM and compound (S)-30 has an $IC_{50}$ value of 0.39 μM while compound (R)-30 has an $IC_{50}$ value of 0.006 μM. Falch shows that the R enantiomer of a compound having a methyl group alpha to the amino moiety of an acyclic cationic side chain possesses better binding characteristics than the S enantiomer of the compound.

11. Given the uncertainties and concerns regarding α-methyl substitution described above in paragraph 9, we were surprised to find that the compounds according to

embodiments of the present invention showed improved metabolic characteristics and retained binding at the α4β2 receptor. As illustrated in Table 5 at Appendix G, compounds according to embodiments of the present invention (Compounds 2 and 3) possesses improved metabolic characteristics when compared with the prior art compounds of Caldwell (Compound 1) as illustrated by their Cp max and AUC values, which are consistently and significantly higher than for the prior art unsubstituted compounds. This improvement in metabolic characteristics was accomplished while retaining acceptable binding at the α4β2 receptor as illustrated by the Ki values in Table 5, which are accurate to approximately a factor of 2.

Given the teachings of Falch, we were also surprised to discover that the S isomers invariably bind with higher affinity (lower Ki) than the R isomers. As illustrated in Table 5, the S isomer of the 5-isopropoxy substituted alpha-methylmetanicotine (Compound 3) has a Ki value of 11 nM compared with a Ki value of 62 nM for the R isomer (Compound 2). The Ki value for Compound 3 is comparable to the Ki value for the corresponding non-α-methyl compound (Compound 1). Moreover, the functional activity of the S isomers at the α4β2 receptor was not only superior to that of the R isomers, but also superior to that of the unsubstituted analogs (see "activity ratio" in Tables 5).

12. Thus, we unexpectedly discovered that the α-methyl compounds according to embodiments of the present invention typically possessed an improved overall combination of biological and pharmacokinetic characteristics (high affinity for the receptor, ability to elicit functional response at the receptor and resistance to metabolic clearance). We more particularly found that the S enantiomers of the α-methyl compounds according to embodiments of the present invention resulted in the best overall combination of biological and pharmacokinetic characteristics. These characteristics make the α-methyl compounds according to embodiments of the present invention significantly better drug candidates than the unsubstituted analogs.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

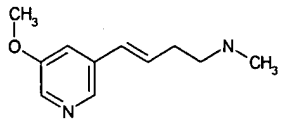
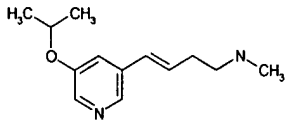
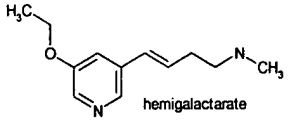
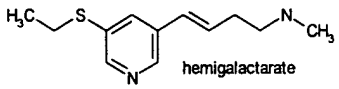
these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

William S. Caldwell

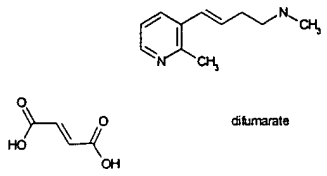
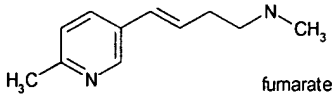
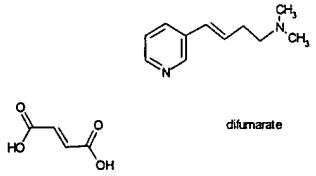
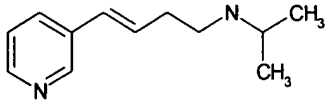
19 Oct, 2001

William S. Caldwell Date

# Table 1

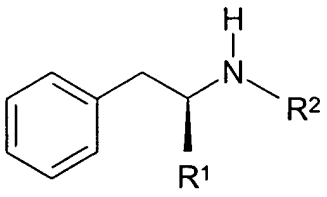
| Compound | STRUCTURE | Ki | Cp max (ng/mL) | AUC 0-∞ (h.ng/mL) |
|---|---|---|---|---|
| 1 |  | 9 | 18 | 23 |
| 2 |  | 5 | 19 | 30 |
| 3 |  hemigalactarate | 5 | 8 | 12 |
| 4 |  hemigalactarate | 28 | 21 | 24 |

# Table 2

| Compound | STRUCTURE | Ki |
|---|---|---|
| 1 |  difumarate | 5585 |
| 2 |  fumarate | 598 |
| 3 |  difumarate | 2067 |
| 4 |  | 270000 |

# Table 3

## Improved Plasma Half-life for β-Phenethylamine Compounds Having an α-Methyl Group

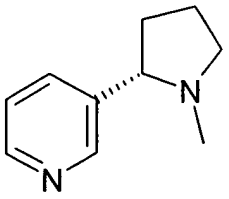



| Species (route) | $R^1$ | $R^2$ | $t_{1/2}$ |
|---|---|---|---|
| Dog (i.v.) | H | H | 5-10 min |
| Dog (i.v.) | H | $CH_3$ | 5-10 min |
| Dog (i.v.) | $CH_3$ | H | 4.5 h |
| Human (i.v.) | $CH_3$ | $CH_3$ | 12.2 h |
| Human (p.o.) | $CH_3$ | $CH_3$ | 10.1 h |

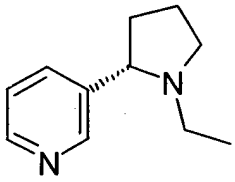
# Table 4

## Effects of Methyl Group Substitution of (S)-(-)-Nicotine on the α4β2 Nicotinic Pharmacophore

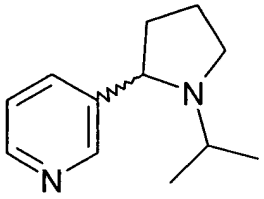
- **Methyl group α to N in (S)-(-)-nicotine**



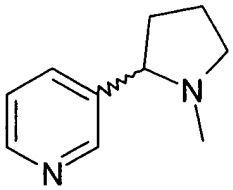
**Ki = 2 nM**



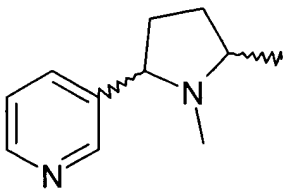
**Ki = 52 nM**



**Ki = 1500 nM**

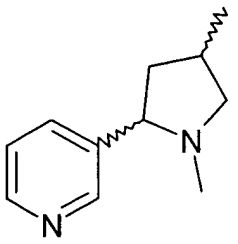


**Ki = 43 nM (Literature value from M.B.)**

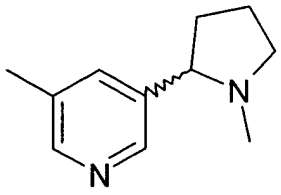


**Ki = 6400 nM**

- **Methyl groups not α to N in (S)-(-)-nicotine**



**Ki = 91 nM**



**Ki = 2 nM**

D. Dell
cc: B Caldwell 10/25/94
P. Luppiello 10/25/94
J. Schmitt

# Synthesis and Evaluation of Nicotine Analogs as Neuronal Nicotinic Acetylcholine Receptor Ligands

Nan-Horng Lin,* George M. Carrera, Jr., and David J. Anderson

*Neuroscience Research, Pharmaceutical Product Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500*

*Received February 22, 1994®*

A series of 3′-, 4′-, and 5′-substituted nicotine analogs have been synthesized and evaluated as ligands of the neuronal nicotinic acetylcholine receptor. The compounds prepared were found to have binding affinities ranging from 4 to 3500 nM. The results indicate that only a small substituent or functionality is well tolerated at the C4′ position of nicotine and that binding affinity is affected by both steric and electronic properties of the substituent. On the other hand, the C3′ and C5′ positions seem to be the more sensitive toward bulky substituents. The best compound, 4′-methylnicotine, is nearly equipotent to nicotine. It possesses the most favorable binding affinity.

## Introduction

Alzheimer's disease, senile dementia of Alzheimer type (AD/SDAT), is a progressive neurodegenerative disorder characterized by a global deterioration of cognitive function, afflicting mainly the elderly.[1] Neurochemical studies of autopsied brain tissue have shown that AD/SDAT is accompanied by multiple changes in numerous brain transmitter systems.[2–5] Although there are a number of neurotransmitter systems affected by Alzheimer's disease, the observed decline in the cholinergic system, and especially a severe depletion of cholinergic neurons, is one hallmark feature of the disease.[6] Moreover, the degree of cognitive impairment in patients with dementia is positively correlated with decreases in markers of cholinergic neuronal function, measured in a postmortem study.[7] More specifically, substantial reductions (30–50%) in nicotinic cholinergic receptors have been consistently reported in the brains of patients with Alzheimer's disease, whereas changes in muscarinic acetylcholine receptors are less remarkable and more dependent on receptor subtype.[8,9] There is currently no totally effective treatment for AD/SDAT, although tacrine has recently been approved by the FDA for use in the Alzheimer's patient, and numerous other clinical trials are underway with agents designed to increase cholinergic tone in the CNS.

Degeneration of the cholinergic neurotransmitter system is not limited to individuals suffering from dementia. Reduction in cholinergic markers in the basal forebrain, decreases in cortical activities of the biosynthetic and degradative enzymes for acetylcholine, decreases in the ability to release acetylcholine from tissue slices, and decreases in numbers of cortical nicotinic acetylcholine receptors have all been reported in otherwise healthy aged individuals.[10] Consistent with these findings are pharmacological studies suggesting that cholinergic deficits are, at least in part, responsible for the memory disturbances in aged animals and humans not suffering from Alzheimer's disease.[11,12]

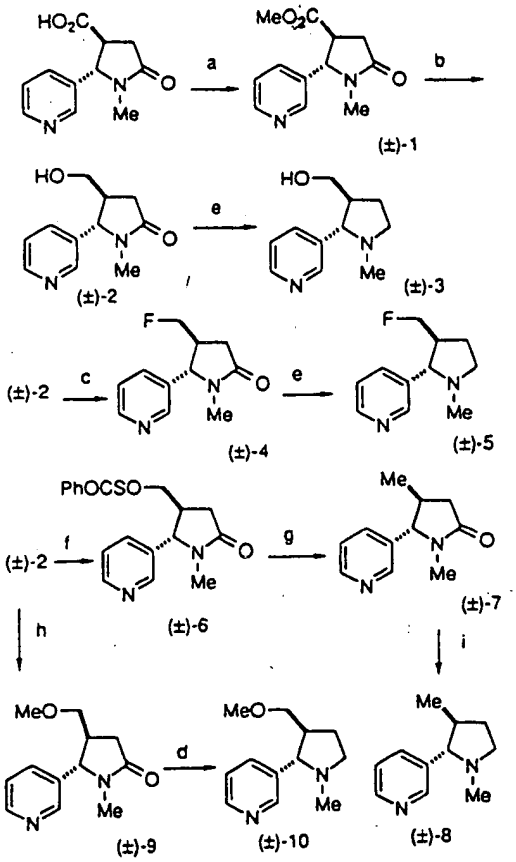
Recent clinical evidence suggests that the characteristic perfusion abnormality observed in Alzheimer's disease patients reflects regional nicotinic cholinergic deficits,[13] and epidemiological evidence suggests a negative correlation between Alzheimer's disease and smoking. Pilot clinical studies suggest that nicotine may be useful for the acute treatment of deficits in attention and information processing associated with Alzheimer's disease.[14,15] These clinical findings are supported by animal studies showing that both acutely- and chronically-administered nicotine enhances cognitive function in rats, an effect that is preserved in aged animals.[16] These results point to the potential of nicotinic agonists for treatment of cholinergic deficits in AD/SDAT. In addition, a neuroregenerative action of chronically-administered nicotine on both neuronal and vascular functions following hemitransection or MPTP-induced destruction of the nigrostriatal dopamine system has also been demonstrated.[17,18]

It may therefore be possible to reverse memory impairment and improve cognitive function of AD/SDAT patients with a nicotinic acetylcholine receptor agonist such as nicotine. It has been demonstrated that chronic nicotine administration to rats enhances cognitive function.[19] Evidence indicates that nicotine might act upon a diverse range of receptor subtype to produce its wide spectrum of behavioral effects. Therefore, it may be possible to design nicotinic acetylcholine agonists which have beneficial effects on learning and memory but, unlike nicotine, do not affect the cardiovascular system nor produce nausea and vomiting. Furthermore, there has been no systematic structure–activity relationship (SAR) on the pyrrolidine ring[20] of nicotine to determine if more potent and/or selective compounds could be prepared for binding to neuronal nicotinic receptors. Therefore, a series of pyrrolidine-modified nicotine analogs have been synthesized with the goal of identifying a compound with an improved pharmacological profile for use as a therapeutic agent for treatment of Alzheimer's disease. We report here the synthesis and the receptor-binding affinity of a series of 3′,4′-disubstituted and 3′-, 4′-, and 5′-substituted nicotine analogs.

* Author to whom the correspondence should be addressed: Nan-Horng Lin, Neuroscience Research Area, D 47W, Abbott Laboratories, Abbott Park, IL 60064-3500. TEL: (708) 938–8797. FAX: (708) 937–9195.

® Abstract published in *Advance ACS Abstracts*, September 1, 1994.

Scheme 1[a]

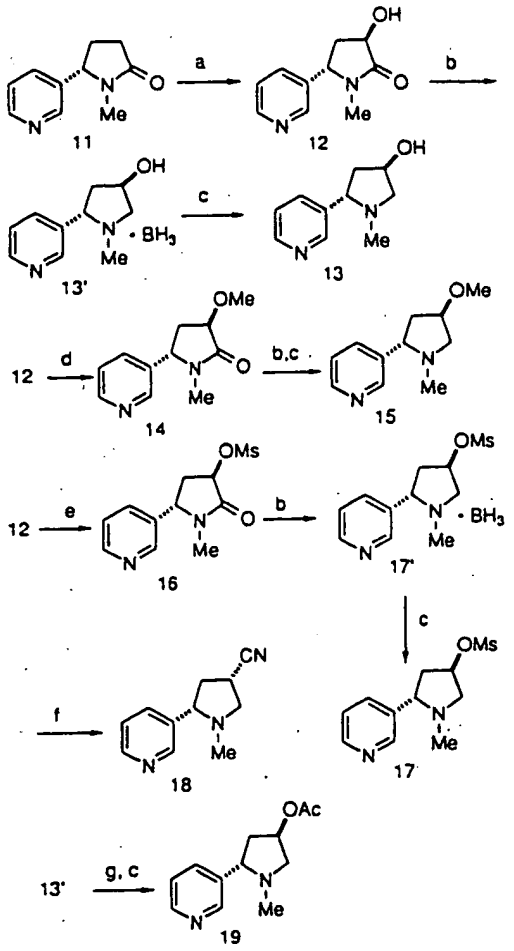


[a] Reagents: (a) HCl, MeOH; (b) $NaBH_4$, MeOH, 0 °C; (c) MsCl, $NEt_3$, then TBAF, THF; (d) $BH_3$, THF, reflux, then CsF, EtOH; (e) $BH_3$, THF, reflux, then $H^+$; (f) ClCSOPh, pyridine; (g) $(Me_3Si)_3SiH$, AIBN; (h) KOH, MeI, DMSO; (i) $BH_3$, THF, reflux, then CsF, dioxane.

## Chemistry

The synthesis of 3′-substituted nicotine analogs is as shown in Scheme 1. Thus, the commercially-available[21] (±)-*trans*-4-cotininecarboxylic acid was esterified with methanol in the presence of hydrochloric acid to give the methyl ester **1**, which was then selectively reduced with sodium borohydride to give (±)-*trans*-4′-(hydroxymethyl)cotinine (**2**). Reduction of **2** with borane in THF followed by borane decomplexation with hydrochloric acid provided the hydoxymethyl analog **3**. The alcohol functionality of **2** was reacted with methanesulfonyl chloride to form the mesylate followed by treatment with tetrabutylammonium fluoride in THF to provide (±)-*trans*-4′-(fluoromethyl)cotinine (**4**). Reduction of **4** with borane in THF followed by borane decomplexation with hydrochloric acid gave the desired substituted nicotine compound **5**. Attempts to synthesize 3′-methylnicotine, following the literature procedure,[22] by treatment of the mesylate with various hydride sources such as lithium aluminum hydride or Superhydride failed to provide the desired methylcotinine in reasonable yield. Thus, the alcohol **2** was reacted with phenyl chlorothionoformate to give compound **6**, which was treated with tris(trimethylsilyl)silane[23] in the presence of AIBN to give 4′-methylcotinine (**7**). This compound was reduced with borane followed by treatment with cesium fluoride, yielding the desired (±)-*trans*-3′-methylnicotine analog (**8**). Treatment of alcohol **2** with potassium hydroxide and methyl iodide in DMSO provided (±)-*trans*-4′-(methoxymethyl)cotinine (**9**) which was then reduced with borane followed by cleavage of the borane complex with cesium fluoride to give (±)-*trans*-3′-(methoxymethyl)nicotine (**10**).

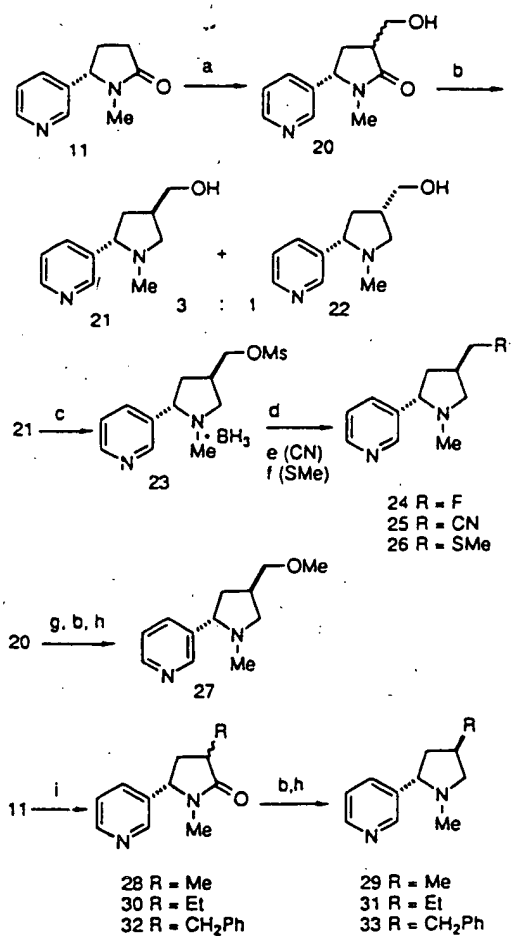
Scheme 2[a]



[a] Reagents: (a) LDA, oxaziridine; (b) $BH_3$, THF; (c) CsF, EtOH, reflux; (d) NaH, MeI, $(Bu)_4NI$; (e) MsCl, $NEt_3$, $CH_2Cl_2$; (f) NaCN, DMSO, $H_2O$; (g) $Ac_2O$, pyridine.

Schemes 2 and 3 outline the preparation of various 4′-substituted nicotine compounds. (*S*)-Cotinine (**11**) was reacted with lithium diisopropylamide (LDA) at −78 °C followed by addition of (+)-(camphorsulfonyl)-oxaziridine and acid workup to give the *trans*-3′-hydroxycotinine (**12**). The $^1H$ NMR spectrum of **12** is identical with that reported in the literature.[24] Compound **12** was reacted with methyl iodide to give the *trans*-3′-methoxycotinine (**14**), which was reduced to the *trans*-4′-methoxynicotine derivative (**15**) with borane/THF as described in Scheme 1. Alternately, *trans*-3′-hydroxycotinine (**12**) was treated with methanesulfonyl chloride in the presence of triethylamine to form the cotinine methanesulfonate **16**, which was reduced with borane in THF to give the nicotine borane complex **17′**. The mesylate **17′** was displaced with any of several nucleophiles to provide various analogs. By displacement with sodium cyanide, the nitrile **18** was formed.[25] It is interesting to note that the borane complex was cleaved in this reaction, leaving the free amine as the product. When *trans*-3′-hydroxycotinine (**12**) was reduced with borane in THF, the *trans*-4′-hydroxynicotine borane complex (**13′**) was formed. Acetylation of compound **13′** with acetic anhydride followed by treatment with cesium fluoride in ethanol gave the *trans*-4′-(acetyloxy)nicotine (**19**).

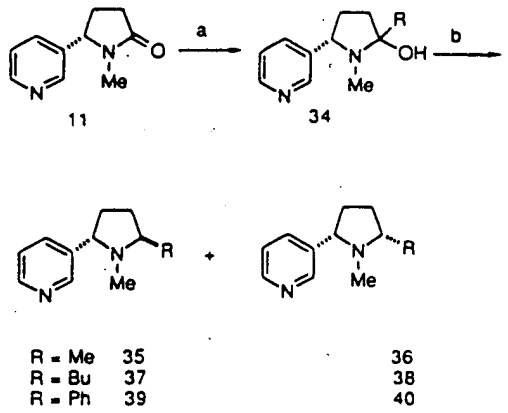
Scheme 3[a]




[a] Reagents: (a) LDA, $H_2CO$, −78 °C; (b) $BH_3$, THF, then CsF, EtOH; (c) $BH_3$, THF, then MsCl, pyridine; (d) TBAF, THF, reflux, 3 h; (e) NaCN, DMSO, $H_2O$; (f) NaSMe, DMF; (g) NaH, MeI, $(n\text{-}Bu)_4NI$; (h) separation of the major isomer; (i) LDA, RBr, THF, −78 °C.

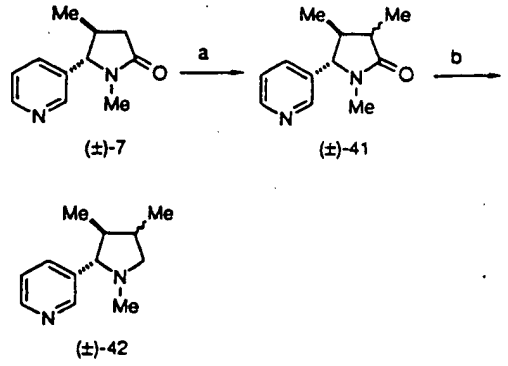
Treatment of (*S*)-cotinine with LDA at −78 °C followed by addition of formaldehyde afforded the 3′-(hydroxymethyl)cotinine (**20**) as a mixture of two diastereoisomers. Reduction of the lactam with borane followed by cleavage of the borane complex with cesium fluoride provided the two (hydroxymethyl)nicotine isomers **21** and **22** in a 3:1 ratio which were readily separated via silica gel column chromatography. The stereochemistry of compounds **21** and **22** were determined by NOE studies and comparing their spectra with those reported in the literature.[26] To avoid any complication in the displacement reactions due to the presence of the free amine functionality, the amino group of compound **21** was protected as the borane complex by reaction with borane in THF. Treatment of the borane complex derived from compound **21** with methanesulfonyl chloride in the presence of triethylamine provided compound **23** which subsequently could be converted to various analogs *via* displacement with nucleophiles. Displacement with fluoride in refluxing THF provided the *trans*-4′-(fluoromethyl)nicotine (**24**); displacement with sodium cyanide in a mixture of DMF and water provided the *trans*-4′-(cyanomethyl)nicotine compound (**25**), whereas displacement with sodium thiomethoxide provided the *trans*-4′-[(methylthio)methyl]nicotine compound (**26**). When compound **20** was treated with sodium hydride followed by methyl iodide in the presence tetrabutylammonium iodide, the corresponding (methoxymethyl)cotinine was formed. It should be noted that this reaction proceeded in very low yield without the presence of the phase transfer agent, tetrabutylammonium iodide. Reduction of the lactam with borane followed by decomplexation with cesium fluoride as described above gave the *trans*-4′-(methoxymethyl)nicotine analog (**27**).

Scheme 4[a]




[a] Reagents: (a) RLi, THF, −78 °C; (b) $NaCNBH_3$, $H^+$.

Scheme 5[a]



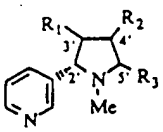

[a] Reagents: (a) LDA, MeI, THF, −78 °C; (b) $BH_3$, THF, reflux, then CsF, EtOH, reflux.

Various 4′-alkyl-substituted nicotine analogs were prepared by treatment of the enolate anion, which was generated by reaction of (*S*)-cotinine with LDA, with the corresponding alkyl halide followed by reduction with borane as shown in Scheme 3.[27]

The 5′-derivatives of nicotine were prepared in accordance with Scheme 4. Following the patent procedure,[28] (*S*)-cotinine (**11**) was reacted with an alkyllithium or phenyllithium in THF at −78 °C to give the amino alcohol intermediate **34**, which could be isolated in pure form via silica gel column chromatography. However, as a general practice, crude **34** was immediately treated with sodium cyanoborohydride in the presence of hydrochloric acid to provide the 5′-alkyl- or 5′-phenylnicotine as a mixture of two diastereoisomers that were readily separated by silica gel column chromatography. The stereochemistry was determined by comparing their $^1H$ NMR and optical rotation data with those reported in the literature.[28]

The 3′,4′-dimethylnicotine derivatives were prepared according to Scheme 5. The (±)-*trans*-4′-methylcotinine (**7**) obtained in Scheme 1 was treated with LDA and methyl iodide to produce the 3′,4′-dimethylcotinine (**41**), which upon reduction with borane/THF as described above was converted to the (±)-3′,4′-dimethylnicotine analog (**42**) as a mixture of two isomers.

Table 1. Binding Data for Pyrrolidine-Modified Nicotine Analogs




| compound[a] | $R_1$ | $R_2$ | $R_3$ | binding affinity,[b,c] $K_i$ (nM) |
|---|---|---|---|---|
| (*S*)-nicotine | H | H | H | 1.15 ± 0.4 |
| 3 | (±)-β-$CH_2OH$ | H | H | 619.2 ± 12.4 |
| 5 | (±)-β-$CH_2F$ | H | H | 98.5 ± 9.7 |
| 8 | (±)-β-Me | H | H | 24.9 ± 1.7 |
| 10 | (±)-β-$CH_2OMe$ | H | H | 2032± 32 |
| 13 | H | (β)-OH | H | 27.6 ± 0.8 |
| 15 | H | (β)-OMe | H | 36.6 ± 0.8 |
| 17 | H | (β)-OMs | H | 363.6 ± 17.9 |
| 18 | H | (α)-CN | H | 82.0 ± 2.0 |
| 19 | H | (β)-OAc | H | 102.9 ± 16.7 |
| 21 | H | (β)-$CH_2OH$ | H | 157.8 ± 7.4 |
| 22 | H | (α)-$CH_2OH$ | H | 294.3 ± 11.0 |
| 24 | H | (β)-$CH_2F$ | H | 11.1 ± 1.9 |
| 25 | H | (β)-$CH_2CN$ | H | 52.0 ± 2.9 |
| 26 | H | (β)-$CH_2SMe$ | H | 492.8 ± 19.2 |
| 27 | H | (β)-$CH_2OMe$ | H | 510.0 ± 46.6 |
| 29 | H | (β)-Me | H | 4.23 ± 0.28 |
| 31 | H | (β)-Et | H | 50.2 ± 1.1 |
| 33 | H | (β)-$CH_2Ph$ | H | 119.4 ± 18.5 |
| 35 | H | H | (β)-Me | 34.9 ± 1.9 |
| 36 | H | H | (α)-Me | 1205.3 ± 34.6 |
| 37 | H | H | (β)-*n*-Bu | 125.2 ± 4.7 |
| 38 | H | H | (α)-*n*-Bu | 1381.4 ± 209.0 |
| 39 | H | H | (β)-Ph | 1242.3 ± 12.4 |
| 40 | H | H | (α)-Ph | 3353.5 ± 196.9 |
| 42 | Me | Me | H | 96.4 ± 6.4 |

[a] Compounds are all enantiomerically pure unless otherwise noted. [b] Values are the means ± SEM. [c] $IC_{50}$ values were converted to $K_i$ values using the Cheng–Prusoff equation as described in the Experimental Section.

## Results and Discussion

The present study evaluates the effects of substitutions at the 3′-, 4′-, and 5′-positions of nicotine which possesses a very high affinity for neuronal nicotinic acetylcholine receptors. The substituents at these positions were varied with respect to size, electronic character, and hydrophobic properties in order to determine the overall effect on ligand-binding affinity. Table 1 shows the $K_i$ value of the 3′-, 4′-, and 5′-substituted and 3′,4′-disubstituted nicotine analogs. The binding procedure is detailed in the Experimental Section.

As shown in Table 1, replacement of the hydrogen at the C3′ position of nicotine with a methyl group (compound 8) decreases the potency by a factor of 22. Replacement of the methyl group with a sterically larger fluoromethyl functionality (i.e., **5**, van der Waal radii, $H$ = 1.2 Å, $F$ = 1.35 Å) results in a further 4-fold decrease in the binding affinity. Changing the substituent to a hydroxymethyl group (compound **3**) reduces the $K_i$ value to 619 nM. Replacement of the hydroxy group of **3** with a methoxy functionality (compound **10**) results in a 3-fold decrease in binding potency. These data are consistent with the hypothesis that binding potency of C3′-substituted analogs is predominantly governed by steric effects.

For 4′-substitution, the present study demonstrates that replacement of hydrogen at the C4′ position of nicotine with a polar hydroxy group (compound **13**) decreases the potency by a factor of 23. Replacement of the hydroxy group with a sterically larger acetoxy functionality (compound **19**) results in a 3.7-fold decrease in binding affinity. Changing this substituent to the sterically much larger (methylsulfonyl)oxy group (**17**) causes a further 3.6-fold decrease. Furthermore, it is demonstrated that extending certain functionalities such as hydroxy or methoxy *via* homologation decreases binding potency (**13** vs **21**, **15** vs **27**). Thus, the same steric effects we observed at the C3′ position has also been shown here. In addition, the same phenomenon has been observed with 4′α-substituted analogs. As shown in Table 1, the nitrile analog **18**, which is sterically smaller than hydroxymethyl, is 3.7-fold more potent than 4′-(hydroxymethyl)nicotine (**22**).

Although a 4′(*R*)-methyl analog, **29**, has comparable binding affinity when compared to (−)-nicotine, deleterious effects are also observed upon substitution with nonpolar groups larger than a methyl group. It is shown from the binding results in Table 1 that the order of binding potency for 4′-alkyl-substituted compounds is Me > Et > benzyl (**29**, **31**, **33**). Replacement of the methyl group with a polar fluoromethyl functionality (i.e., **24**) results in a 2.5-fold decrease in the binding affinity. Changing the substituent to a hydroxymethyl group (**21**) reduced the $K_i$ value to 158 nM (14-fold decrease). Replacement of the hydroxy group of **21** with a methoxy functionality (i.e., **27**) results in a further 3.2-fold decrease in binding potency. The deleterious effect could be due to steric occlusion in this region of the receptor. The data in Table 1 indicate that steric volume of a methyl group may represent an upper limit which may be accommodated by the space in the receptor ligand-binding domain since a large decrease in binding was observed in going from a methyl to ethyl group. Thus, steric factors are clearly important for optimal binding potency for the substituents at the C4′

position of the pyrrolidine ring. However, it is noted that the 4′-benzyl analog **33**, which is sterically larger than the 4′-methoxymethyl analog **27**, is 4.3-fold more potent. In addition, further substitution with either an acetoxy (**19**) or cyanomethyl (**25**) group causes a less dramatic loss of affinity than with the smaller hydroxymethyl group (**21**). Therefore, electronic effects may play at least a minor role in influencing binding affinities.

Although the effect of stereochemistry of substituents on the pyrrolidine ring has not been reported in the literature, it is interesting to note that the configuration at the C4′ position has only a small effect on activity. Specifically, the C4′ epimer of (*R*)-(hydroxymethyl)-nicotine **21**, (*S*)-(hydroxymethyl)nicotine **22**, is 2-fold less potent than **21**. However, the limited number of compounds with α stereochemistry at the C4′ position makes it difficult to draw any conclusions regarding the effect of stereochemistry on SAR.

The effect of substitution on binding affinity at the C5′ position was also examined. Replacement of the C5′ hydrogen of nicotine with an alkyl or phenyl group results in a decrease in binding potency ranging by factors of 35−3353. Specifically, the potency of the β-methyl analog **35** is reduced by a factor of 35, and the β-butyl analog **37** has an affinity over 125-fold lower than that of nicotine. Changing the substituent to a β-phenyl group (**39**) led to a 1240-fold drop in potency. These results suggest that any β substitution at this position is not well tolerated. Likewise, C5′α substituents are not well tolerated, with the α-methyl analog **36** demonstrating greater than 1000-fold decrease in binding affinity compared to that of (−)-nicotine. This unfavorable steric interaction appears to be more severe in the C5′α-substituted series than in the C5′β-substituted series, a phenomenon not observed with C4′-substituted analogs. Thus the $K_i$ value of the α-methyl isomer **36** is 30-fold greater than that of the β isomer **35**, and the $K_i$ value of the α-butyl analog **38** is over 10-fold higher than that of the β-butyl analog **37**. Although the substituents at the C5′ position might change the bioactive conformation of the molecule, the reduction in potency might also result from the steric interaction between the α-alkyl substituents and the nicotinic acetylcholine receptor. It has been shown by NMR analysis[29] that nicotine in solution exists primarily in a conformation in which the methyl group on the pyrrolidine ring is trans to the pyridine ring; the pyrrolidine ring is in an envelope conformation, and the relative orientation of pyrrolidine and pyridine rings is orthogonal. Using this NMR conformational analysis as a starting point, we have carried out molecular mechanics calculations[30] to understand the binding potency difference between α and β isomers. However, our calculations failed to provide any conclusive results. Since the bioactive conformation of 5′-substituted analogs is not known at the present time, we assumed that either steric or conformational effects or a combination of both may account for the observed result. Since the C5′ position is directly adjacent to the pyrrolidine nitrogen atom, which is believed to constitute a critical binding point,[31] close contact with the protein in this region is quite plausible. The effect of stereochemistry of substituents on binding affinity will require additional studies.

We have also demonstrated that the substitution position of a functional group has dramatic effects on binding affinity. Hence, the 5′β-methylnicotine analog (**35**) is significantly less potent in binding than its 4′-substitution counterpart, **29**, while the 3′-methylnicotine analog (**8**) exhibits intermediate activity. However, it should be noted that the 3′-substituted analogs examined in this study are all racemates. Thus, these analogs may possess affinity even higher that those shown here had each enantiomer been synthesized and evaluated. The 3′,4′-dimethyl analog **42** exhibits weaker potent binding affinity than either compound **8** or **29**. The reduction in potency might result from the steric interaction between the methyl substituents and the nicotinic acetylcholine receptor.

In conclusion, pyrrolidine-modified nicotine analogs possessing various substituents at the 3′-, 4′-, and 5′-positions have been synthesized and evaluated for binding potency to the neuronal nicotinic acetylcholine receptor. The SAR generated has provided valuable information concerning the structural requirements of the pyrrolidine ring of nicotine and its analogs. Although it has been demonstrated that the nicotinic acetylcholine channel protein possesses five subunits,[32] the makeup of which may complicate the interpretation of SAR data, these data can be used to help begin to define the volume of available space in the pyrrolidine ring region of the receptor ligand-binding domain. On the basis of this initial information, novel nicotinic acetylcholine ligands are currently being synthesized to further characterize the nicotinic acetylcholine receptor binding domain and the ligand receptor interaction.

## Experimental Section

Proton magnetic resonance spectra were obtained on a Nicolet QE-300 (300 MHz) and a General Electric GN-300 (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) relative to $Me_4Si$ as an internal standard unless otherwise indicated. Mass spectra were obtained with a Hewlett Packard HP5965 spectrometer. Elemental analyses and the above determinations were performed by the Analytical Research Department, Abbott Laboratories.

Thin-layer chromatography (TLC) was carried out by using E. Merck precoated silica gel F-254 plates (thickness 0.25 mm). Flash chromatography was carried out using Merck silica gel 60, 200−400 mesh.

Melting points are uncorrected and were determined on a Buchi melting point apparatus. Optical rotation data were obtained on a Perkin-Elmer Model 241 polarimeter. All reactions were performed under anhydrous conditions unless otherwise noted. The following abbreviations are used in the Experimental Section: THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; $D_2O$, deuterium oxide; $CDCl_3$, deuteriochloroform, DMSO-$d_6$, deuteriodimethyl sulfoxide; BOC, *tert*-butyloxycarbonyl; CBZ, benzyloxycarbonyl; Bn, benzyl; Ms, methanesulfonyl; PAW, pyridine/acetic acid/water (20:6:11); DCC, dicyclohexylcarbodiimide; DIBAL-H, diisobutylaluminum hydride; DIEA, diisopropylethylamine; DPPA, diphenyl phosphorazidate; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; EtOH, ethanol; IBCF, isobutyl chloroformate; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; LAH, lithium aluminum hydride; $NH_4OAc$, ammonium acetate; NMM, *N*-methylmorpholine.

**(±)-4′-*trans*-(Hydroxymethyl)cotinine (2).** A sample of methyl *trans*-4-cotininecarboxylate (557 mg, 2.38 mmol) (prepared from the acid, which is available from Aldrich Chemical Co.) in methanol (20 mL) was cooled to 0 °C. Sodium borohydride (135 mg, 3.57 mmol) was added portionwise to the reaction mixture under nitrogen at 0 °C. After stirring for 10 min at 0 °C, the reaction mixture was warmed to room

temperature and allowed to stir for an additional 2 h. After the reaction was completed, it was quenched by addition of saturated aqueous sodium bicarbonate solution. The desired product was extracted into chloroform from water by a continuous extraction method. The solvent was removed under reduced pressure to give a light yellow oil which was chromatographed on silica gel, eluting with chloroform/methanol (10:1), to provide the title compound (477 mg, 97% yield) as a colorless oil. MS (DCI/$NH_3$): *m/z* 207 $(M + H)^+$, 224 $(M + NH_3)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 2.30–2.41 (m, 2H), 2.49 (s, 3H), 2.65–2.77 (m, 1H), 3.70–3.76 (m, 2H), 4.49 (d, $J$ = 6 Hz, 1H), 7.35 (dd, $J$ = 4.5, 9.0 Hz), 7.55 (dt, $J$ = 2.5, 9.0 Hz, 1H), 8.53 (d, $J$ = 2.5 Hz, 1H), 8.61 (dd, $J$ = 2.5, 4.5 Hz, 1H).

**(±)-3′-*trans*-(Hydroxymethyl)nicotine (3).** Compound 2 (640 mg, 3.10 mmol) in THF (20 mL) was treated dropwise with a 1 M solution of borane (9.3 mL, 9.3 mmol) in THF at room temperature. After the mixture was refluxed for 3 h, the reaction was quenched by addition of a large excess of methanol. The resultant solution was allowed to stir at room temperature for an additional 15 min. Solvent was then removed under reduced pressure to give a white solid. The crude reaction product was dissolved in methanol (12 mL) and treated with 6 N aqueous hydrochloric acid (0.4 mL). After the pH of the solution was adjusted to 2.0 by addition of 15% aqueous sodium hydroxide solution, the solvent was concentrated *in vacuo*. The resultant crude product was purified by flash column chromatography on silica gel, eluting with chloroform/methanol (10:1), to give 343 mg of the title compound (58%) as a colorless oil. MS (DCI/$NH_3$): *m/z* 193 $(M + H)^+$, 210 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.65–1.80 (m, 1H), 2.16 (s, 3H), 2.10–2.48 (m, 2H), 2.94 (br d, $J$ = 8 Hz, 1H), 3.26 (br t, $J$ = 8.0 Hz, 1H), 3.55–3.70 (m, 2H), 7.29 (dd, 1H, $J$ = 2.5, 5.0 Hz, overlap with $CDCl_3$), 7.55 (m, 1H), 8.51 (d, $J$ = 2.5, 5.0 Hz, 1H), 8.54 (d, $J$ = 2.5 Hz, 1H). Anal. ($C_{11}H_{14}N_2O$·0.55$H_2O$) C, N; H: calcd, 7.60; found, 8.05.

**(±)-4′-*trans*-(Fluoromethyl)cotinine (4).** In a 50 mL round-bottomed flask equipped with a rubber septum and a magnetic stir bar were placed 2 (363 mg, 1.76 mmol) and dichloromethane (20 mL). To this stirring solution, at room temperature, was added triethylamine (0.248 mL, 1.94 mmol) followed by methanesulfonyl chloride (0.164 mL, 2.11 mmol). The reaction mixture was stirred for 30 min and then the reaction quenched with methanol to destroy excess methanesulfonyl chloride. The reaction mixture was concentrated with a rotary evaporator to obtain a dark yellow oil. This crude material (499 mg, 1.76 mmol) was placed in a 25 mL round-bottomed flask, and 1 M tetra-*n*-butylammonium fluoride (7.39 mL, 7.39 mmol) in THF was added to the system. This solution was refluxed under nitrogen for 30 min. The reaction mixture was concentrated, and the residue was taken up with chloroform. After washing with saturated aqueous sodium bicarbonate solution, the organic layer was dried and concentrated under reduced pressure to give a yellow oil. The crude material was purified by flash column chromatography (50 g of silica gel), eluting with chloroform/methanol (100:3), to provide 131 mg (36% yield) of the title compound as a light yellow oil. MS (DCI/$NH_3$): *m/z* 209 $(M + H)^+$, 226 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 2.36 (d, $J$ = 9.0, 15 Hz, 1H), 2.42–2.58 (m, 1H), 2.71 (s, 3H), 2.74 (dd, $J$ = 9.0, 15 Hz, 1H), 4.52 (d, $J$ = 5.2 Hz, 1H), 4.48 (ddd, $J$ = 2.6, 5.1, 47 Hz, 2H), 7.47 (dd, $J$ = 5.2, 7.7 Hz, 1H), 7.66 (dt, $J$ = 1.9, 8.1 Hz, 1H), 8.57 (d, $J$ = 1.1 Hz, 1H), 8.66 (m, 1H).

**(±)-3′-*trans*-(Fluoromethyl)nicotine Oxalate (5).** Following the same procedure as described in the preparation of 3, compound 4 (131 mg, 0.62 mmol) in THF (10 mL) was treated with borane (1.25 mL, 1.25 mmol) to give 59 mg of the title compound (49%) as a colorless oil. MS (DCI/$NH_3$): *m/z* 195 $(M + H)^+$, 212 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.70–1.82 (m, 1H), 2.15 (s, 3H), 2.16–2.20 (m, 1H), 2.32–2.45 (m, 2H), 2.97 (d, $J$ = 9 Hz, 1H), 3.24 (t, $J$ = 9.0 Hz, 1H), 4.47 (ddd, $J$ = 4.5, 9.0, 48 Hz, 2H), 7.28 (m, 1H, overlap with $CDCl_3$), 7.72 (dt, $J$ = 2.5, 9.0 Hz, 1H), 8.53 (dd, $J$ = 2.5, 5.0 Hz, 1H), 8.57 (d, $J$ = 2.5 Hz, 1H).

To the solution of the product obtained from above in diethyl ether (1.5 mL) was added oxalic acid in diethyl ether (5 mL) dropwise at 0 °C. After the mixture was stirred at 0 °C for 15 min, the precipitate was collected by centrifugation, washed with diethyl ether three times, and dried *in vacuo* to yield the title compound as a white powder. Mp: 86–89 °C. MS (DCI/$NH_3$): *m/z* 195 $(M + H)^+$, 212 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): $\delta$ 2.18–2.32 (m, 1H), 2.41–2.58 (m, 1H), 2.82 (s, 3H), 3.07–3.30 (m, 1H), 3.36–3.51 (m, 1H), 4.38–4.74 (m, 2H, overlap with $D_2O$ peak), 4.85–4.98 (m, 1H), 7.76 (dd, $J$ = 5.0, 7.5 Hz, 1H), 8.26 (d, $J$ = 7.5 Hz, 1H), 8.75 (d, $J$ = 5.0 Hz, 1H), 8.79 (s, 1H). Anal. ($C_{11}H_{15}N_2F$·1.6$C_2H_2O_4$·1.0$H_2O$) C, H, N.

**(±)-4′-*trans*-[[[Phenoxy(thiocarbonyl)]oxy]methyl]-cotinine (6).** In a 50 mL round-bottomed flask equipped with a rubber septum and a magnetic stir bar were placed 4-(hydroxymethyl)cotinine (2) (468 mg, 2.27 mmol) and dichloromethane (15 mL). To this stirring solution, at room temperature, was added pyridine (0.733 mL, 9.0 mmol) followed by chlorophenoxythiocarbonate (0.373 mL, 2.72 mmol). The reaction mixture was stirred at room temperature for 2 h and at 0–5 °C for 19 h. The reaction was then quenched with methanol to destroy excess chloride. The reaction mixture was concentrated with a rotary evaporator to obtain a dark yellow oil. This crude material was subjected to flash column chromatography (50 g of silica gel), gradually increasing the polarity of the eluent from 2:1 hexane/acetone to 1:1 hexane/acetone, to obtain 576 mg (74% yield) of the phenoxythiocarbonate as a white solid. MS (DCI/$NH_3$): *m/z* 343 $(M + H)^+$, 360 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 2.43 (dd, $J$ = 6, 15 Hz, 1H), 2.68–2.89 (m, 2H), 2.72 (s, 3H), 4.54–4.62 (m, 2H), 4.49 (d, $J$ = 6.0 Hz, 1H), 7.06–7.12 (m, 1H), 7.31–7.35 (m, 1H), 7.36–7.48 (m, 3H), 7.59 (dt, $J$ = 8.5, 2 Hz, 1H), 8.57 (s, 1H), 8.65 (m, 1H).

**(±)-4′-*trans*-Methylcotinine (7).** To a solution of the compound 6 (392 mg, 1.14 mmol) in toluene (15 mL) containing azobis(isobutyronitrile) (30 mg, 0.38 mmol) was added tris(trimethylsilyl)silane (0.52 mL, 1.72 mmol). The resultant solution was degassed under nitrogen. After 2 h at 90 °C, the toluene was removed under reduced pressure and the residue was allowed to stand on a silica gel column for 30 min prior to elution with chloroform/methanol, 100:7. There was obtained 246 mg (76%) of the title compound as a colorless oil. MS (DCI/$NH_3$): *m/z* 191 $(M + H)^+$, 208 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.19 (d, $J$ = 7.5 Hz, 3H), 2.12–2.26 (m, 2H), 2.67 (s, 3H), 2.69–2.83 (m, 1H), 4.11 (d, $J$ = 6.0 Hz, 1H), 7.54 (m, 1H), 7.74 (d, $J$ = 7.5 Hz, 1H), 8.58 (s, 1H), 8.65 (d, $J$ = 4.5 Hz, 1H).

**(±)-3′-*trans*-Methylnicotine Dioxalate (8).** Compound 7 (112 mg, 0.59 mmol) in THF (6 mL) was treated dropwise with a 1 M solution of borane (1.76 mL, 1.76 mmol) in THF at room temperature. After the solution was refluxed for 2 h, the reaction was quenched by addition of a large excess of methanol. The resultant solution was allowed to stir at room temperature for an additional 15 min. Solvent was then removed under reduced pressure to give a white solid. The crude reaction product (110 mg) was dissolved in a mixture of dioxane (3 mL) and ethanol (6 mL). This reaction mixture was then treated with cesium fluoride (204 mg, 1.76 mmol) and refluxed overnight. The crude product was purified by flash column chromatography on silica gel, eluting with hexane/acetone (1:1), to give 22 mg (21% yield for two steps) of the title compound as a colorless oil. MS (DCI/$NH_3$): *m/z* 177 $(M + H)^+$, 194 $(M + NH_3)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 0.98 (d, $J$ = 6.0 Hz, 1H), 1.41–1.59 (m, 1H), 1.55–1.79 (m, 1H), 2.16 (s, 3H), 2.33–2.50 (m, 1H), 2.59–2.72 (m, 1H), 3.20–3.40 (m, 1H), 3.68–3.74 (m, 1H), 7.25–7.34 (m, 1H, overlap with $CDCl_3$ peak), 7.67–7.83 (m, 1H), 8.50–8.59 (m, 2H).

To the solution of the product obtained above in diethyl ether (1.5 mL) was added oxalic acid in diethyl ether dropwise at 0 °C. After stirring at 0 °C for 15 min, the precipitate was collected by centrifugation, washed with diethyl ether three times, and dried *in vacuo* to yield the title compound as a white powder. Mp: 98–101 °C. MS (DCI/$NH_3$): *m/z* 177 $(M + H)^+$, 194 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): $\delta$ 1.05 (d, $J$ = 6.6 Hz, 3H), 1.93–2.09 (m, 1H), 2.41–2.55 (m, 1H), 2.70–2.90 (m, 1H, overlap with 2.83 peak), 2.83 (s, 3H), 3.36–3.52 (m, 1H), 3.84–4.03 (m, 1H), 4.21 (d, $J$ = 9.0 Hz, 1H), 8.01 (dd, $J$ = 5.5, 9.0 Hz, 1H), 8.49–8.58 (m, 1H), 8.86 (dd, $J$ = 1.5, 5.5 Hz, 1H), 8.90 (d, $J$ = 3 Hz, 1H). Anal. ($C_{11}H_{16}N_2$·2.4$C_2H_2O_4$) C, H, N.

**(±)-4′-*trans*-(Methoxymethyl)cotinine (9).** A sample of 3-*trans*-(hydroxymethyl)cotinine (143 mg, 0.69 mmol) was dissolved in DMSO (1.5 mL) containing potassium hydroxide (154 mg, 2.76 mmol) and stirred at room temperature for 15 min. Methyl iodide (0.086 mL, 1.38 mmol) was then added to the reaction mixture, and after stirring at room temperature for 1 h, the solvent was evaporated under reduced pressure. The crude product was purified on a flash silica gel column, eluting with chloroform/methanol (10:1), to give 66 mg (43%) of the title compound. MS (DCI/$NH_3$): *m/z* 221 $(M + H)^+$, 238 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): δ 2.46–2.58 (m, 3H), 2.69 (s, 3H), 3.38 (s, 3H), 3.42 (d, *J* = 6 Hz, 2H), 4.43 (d, *J* = 6 Hz, 1H), 7.37 (dd, *J* = 4.5, 6 Hz, 1H), 7.56 (m, 1H), 8.52 (m, 1H), 8.61 (m, 1H).

**(±)-3′-*trans*-(Methoxymethyl)nicotine Dioxalate (10).** Compound **9** (66 mg, 0.30 mmol) was treated with borane followed by cesium fluoride as described in the preparation of compound **8**. The crude product was purified by flash chromatography on silica gel, eluting with acetone/hexane (1:1), to give 36 mg (23%) of the free base. By the procedure described in **8**, the product described above was converted to the oxalate salt in quantitative yield to give 64 mg of the title compound as a very hygroscopic salt. MS (DCI/$NH_3$): *m/z* 207 $(M + H)^+$, 224 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): δ 2.05–2.19 (m, 1H), 2.40–2.55 (m, 1H), 2.83 (s, 3H), 3.01–3.16 (m, 1H), 3.22 (s, 3H), 3.40–3.53 (m, 1H), 3.56 (dd, *J* = 3.0, 6.0 Hz, 1H), 3.93 (br s, 1H), 4.88 (m, 1H), 4.52 (m, 1H), 8.06 (dd, *J* = 5, 8.1 Hz, 1H), 8.61 (m, 1H), 8.87 (d, *J* = 5.6 Hz, 1H), 8.96 (m, 1H). Anal. ($C_{12}H_{18}N_2O{\cdot}1.5C_2H_2O_4$) C, H, N.

**(3′*R*,5′*S*)-3′-Hydroxycotinine (12).** A sample of (*S*)-cotinine (1.2 g, 6.8 mmol; from Aldrich Chemical Co.) was dissolved in THF (30 mL) and cooled to −78 °C. LDA solution (1.5 M in hexane, 13.6 mmol) was added, and the solution was stirred and warmed to 0 °C for 30 min. The solution was cooled to −78 °C, and (+)-(camphorylsulfonyl)oxaziridine (2.5 g, 10.9 mmol) dissolved in THF (24 mL) was added. The reaction mixture was stirred for 2 h and the reaction quenched by addition of methanol. This mixture was stirred for 15 min, and the solvent was removed. The residue was subjected to flash chromatography on silica gel using chloroform/methanol (100:7) as eluent. The title compound was isolated as an oil (1.1 g, 84% yield). $[\alpha]_D$ +39° (*c* 0.48, MeOH) (lit.[24] $[\alpha]_D$ +42.2° (*c* 2.5, MeOH). MS (DCI/$NH_3$): *m/z* 193 $(M + H)^+$. $^1H$ NMR ($CDCl_3$): δ 2.34 (ddd, *J* = 13.5, 9.0, 3.0 Hz, 1H), 2.51 (m, 1H), 2.78 (s, 3H), 4.57 (t, *J* = 7.5 Hz, 1H), 4.66 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.45 (m, 1H), 7.35 (dd, *J* = 9.0, 6.0 Hz), 8.61 (dd, *J* = 5.6, 3 Hz, 1H), 8.49 (d, *J* = 3 Hz, 1H).

**(2′*S*,4′*R*)-4′-Hydroxynicotine Dioxalate (13).** A 1 M solution of borane (3.71 mL, 3.71 mmol) in THF was added dropwise over a period of 5 min to compound **12** (357 mg, 1.86 mmol) in THF (2 mL) under nitrogen. After the mixture was refluxed for 2 h, methanol was added dropwise and the reaction mixture stirred for an additional 15 min. The solvent was then removed *in vacuo*, affording a white solid borane complex. This solid was dissolved in anhydrous ethanol, cesium fluoride (1.30 g, 11.16 mmol) was added, and the resultant solution was refluxed overnight. Evaporation of the solvent provided a white solid which was purified on a silica gel column, eluting with chloroform/methanol (10:1), to give 105 mg of the desired alcohol as an oil in 32% yield. MS (DCI/$NH_3$): *m/z* 179 $(M + H)^+$. $^1H$ NMR ($CDCl_3$): δ 2.03–2.10 (m, 2H), 2.18 (s, 3H), 2.33 (dd, *J* = 5.2, 10 Hz, 1H), 3.52 (dd, *J* = 7.3, 9.5 Hz), 3.59 (dd, *J* = 6.7, 10.3 Hz, 1H), 4.47 (m, 1H), 7.43 (dd, *J* = 5.2, 7.7 Hz, 1H), 7.85 (dt, *J* = 5.9, 1.8 Hz, 1H), 8.45 (dd, *J* = 5.2, 1.5 Hz, 1H), 8.50 (d, *J* = 1.5 Hz, 1H).

A solution of the amine obtained from above (34 mg, 0.19 mmol) in ethanol was added dropwise to a stirred solution of oxalic acid (25 mg, 0.28 mmol) in diethyl ether at room temperature. The resultant white precipitate was then collected by centrifugation and triturated with three portions of diethyl ether. The hygroscopic solid was obtained in 50% yield (25.4 mg). Mp: 208–211 °C. $[\alpha]_D$ −15.5° (*c* 0.11, MeOH). MS (DCI/$NH_3$): *m/z* 179 $(M + H^+)$, 196 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): δ 2.55 (dd, *J* = 6, 13 Hz), 2.73 (m, 2H), 2.96 (s, 3H), 3.37 (m, 1H), 4.19 (m, 1H), 4.93 (m, 1H), 7.82 (m, 1H); 8.32 (d, *J* = 9 Hz, 1H), 8.71 (d, *J* = 6 Hz), 8.82 (s, 1H). Anal. ($C_{10}H_{14}N_2O{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

**(3′*R*,5′*S*)-3′-Methoxycotinine (14).** A sample of hydroxycotinine (80 mg, 2 mmol) was dissolved in THF containing sodium hydride and stirred at room temperature for 15 min. Tetrabutylammonium iodide (20 mg, 0.05 mmol) and methyl iodide (0.020 mL, 0.32 mmol) were then added to the reaction mixture. After stirring at room temperature for 20 h, the solvent was evaporated under reduced pressure. The crude product was purified on a flash silica gel column. Elution with chloroform/methanol (20:1) gave 17 mg (53%) of the title compound. MS (DCI/$NH_3$): *m/z* 207 $(M + H)^+$, 224 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): δ 2.14–2.26 (m, 1H), 2.42–2.55 (m, 1H), 2.73 (s, 3H), 3.32–3.43 (m, 3H), 3.58 (s, 3H), 4.12 (m, 1H), 4.66 (m, 1H), 7.36 (m, 1H), 7.47 (m, 1H), 8.5 (m, 1H), 8.62 (m, 1H).

**(2′*S*,4′*R*)-4′-Methoxynicotine Dioxalate (15).** Compound **14** (170 mg, 0.83 mmol) was treated with borane followed by cesium fluoride as described in the preparation of **13**. The crude product was purified by flash chromatography on silica gel, eluting with acetone/hexane (1:1), to give 36 mg (23% yield for two steps). MS (DCI/$NH_3$): *m/z* 191 $(M + H)^+$, 208 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): δ 1.4–1.52 (m, 1H), 1.88–197 (m, 2H), 2.09–2.12 (m, 1H), 2.15 (s, 3H), 2.6–2.7 (m, 1H), 3.13–3.41 (m, 2H), 3.37 (s, 3H), 7.25–7.29 (m, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 8.51 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.53 (d, *J* = 1.5 Hz, 1H).

By the procedure described in the preparation of salt **13**, the product obtained above was converted to the oxalate salt in quantitative yield to give 64 mg of the title compound as a very hygroscopic salt. Mp: 150–152 °C. $[\alpha]_D$ −2.56° (*c* 0.19, MeOH). MS (DCI/$NH_3$): *m/z* 193 $(M + H)^+$, 210 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): δ 2.60–2.70 (m, 1H), 2.74–2.81 (m, 1H), 2.92 (s, 3H), 3.42 (s, 3H), 3.46–3.55 (m, 1H), 4.25 (m, 1H), 4.88 (m, 1H), 4.44 (m, 1H), 8.09 (dd, *J* = 5, 8.1 Hz, 1H), 8.67 (d, *J* = 8.1 Hz, 1H), 8.89 (d, *J* = 5.6 Hz, 1H), 8.99 (s, 1H). Anal. ($C_{11}H_{16}N_2O{\cdot}2.8C_2H_2O_4{\cdot}H_2O$) C, H, N.

**(3′*R*,5′*S*)-3′-[(Methylsulfonyl)oxy]cotinine (16).** In a 50 mL round-bottomed flask equipped with a rubber septum and a magnetic stir bar were placed product **12** (554 mg, 2.89 mmol) and dichloromethane (20 mL). To this stirring solution, at room temperature, was added triethylamine (0.59 mL, 4.62 mmol) followed by methanesulfonyl chloride (0.34 mL, 4.34 mmol). The reaction mixture was stirred for 19 h and then the reaction quenched with methanol to destroy excess methanesulfonyl chloride. The reaction mixture was concentrated with a rotary evaporator to obtain a dark yellow oil. This crude material was subjected to flash chromatography (50 g of silica gel), gradually increasing the polarity of the eluent from 100:5 chloroform/methanol to 100:7 chloroform/methanol, to obtain 420 mg (57% yield) of the methanesulfonate ester as a pale yellow viscous oil. MS (DCI/$NH_3$): *m/z* 257 $(M + H)^+$, 264 $(M + NH_4)^+$. $^1H$ NMR ($CD_3OD$): δ 2.24 (s, 3H), 2.65–2.61 (m, 2H), 3.65–3.74 (m, 1H), 3.07 (s, 3H), 3.75–3.85 (m, 1H), 4.25–4.32 (m, 2H), 5.24–5.34 (m, 1H), 7.62 (dd, *J* = 6, 7.5 Hz, 1H), 8.09 (d, *J* = 7.5 Hz, 1H), 8.51 (d, *J* = 6 Hz, 1H), 8.59 (s, 1H).

**(2′*S*,4′*R*)-4′-[(Methylsulfonyl)oxy]nicotine Dioxalate (17).** To compound **16** (652 mg, 2.41 mmol) in THF (15 mL) was added under nitrogen and dropwise over a period of 5 min a 1 M solution of borane (6.03 mL, 6.03 mmol) in THF. After the mixture was stirred under reflux for 2 h, methanol was added dropwise and the reaction mixture stirred for an additional 1 h. The solvent was then removed *in vacuo*, affording a white solid borane complex, **17′**. A sample of the borane complex (312 mg) was dissolved in anhydrous ethanol, cesium fluoride (334 mg) was added, and the resultant solution was refluxed overnight. Evaporation of the solvent provided a white solid which was purified on a silica gel column, eluting with chloroform/methanol (10:7) to give 117 mg of the desired mesylate. Following the procedure for the preparation of salt **13** above, a 35 mg sample of the oxalate salt was prepared. Mp: 122–125 °C. $[\alpha]_D$ −6.55° (*c* 0.28, $CHCl_3$). MS (DCI/$NH_3$): *m/z* 257 $(M + H)^+$. $^1H$ NMR ($D_2O$): δ 2.85 (m, 5H), 3.35 (s, 3H), 3.77 (d, *J* = 14 Hz, 1H), 4.38 (dd, *J* = 5.5, 13, 1H), 5.0

(m, 1H), 5.7 (m, 1H), 7.87 (m, 1H), 8.37 (dt, $J$ = 8, 1.5 Hz, 1H), 8.79 (m, 1H), 8.82 (m, 1H). Anal. ($C_{11}H_{16}N_2O_3S \cdot 1.7C_2H_2O_4$) C, H, N.

**(2′*S*,4′*S*)-4′-Cyanonicotine Dioxalate (18).** The amine borane complex **17′** (100 mg, 0.39 mmol) was dissolved in DMF (4 mL) and sodium cyanide (190 mg) was added. This solution was heated at 105 °C under nitrogen for 16 h. The reaction mixture was concentrated with a rotary evaporator, and the residue was taken up in chloroform. The crude material was purified by flash chromatography (50 g of silica gel), eluting with chloroform/methanol (100:0.7), to provide 24 mg (33% yield) of the title compound as a yellow oil. MS (DCI/$NH_3$): $m/z$ 188 $(M + H)^+$, 205 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 2.02–2.13 (m, 1H), 2.21 (s, 3H), 2.62–2.72 (m, 1H), 3.06–3.16 (m, 1H), 3.21–3.31 (m, 1H), 3.51 (d, $J$ = 9.2 Hz, 1H), 7.41 (dd, $J$ = 4.8, 7.7 Hz, 1H), 7.94 (d, $J$ = 8.1 Hz, 1H), 8.53–8.60 (m, 2H).

The oil from above was dissolved in diethyl ether to which was added, dropwise, a solution of oxalic acid (13 mg, 0.14 mmol) in diethyl ether. The resultant precipitate was collected by centrifugation to give 37 mg of the title compound. Mp: 130–133 °C. $[\alpha]_D$ −50.4° ($c$ 0.10, MeOH). MS (DCI/$NH_3$): $m/z$ 188 $(M + H)^+$, 205 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): $\delta$ 2.60 (s, 3H), 2.60–2.70 (m, 1H), 3.02–3.22 (m, 1H), 3.51 (dd, $J$ = 9.2, 12.1 Hz, 1H), 3.75–3.88 (m, 1H), 3.99 (dd, $J$ = 3.6, 11.7 Hz, 1H), 4.37 (dd, $J$ = 7.7, 10.0 Hz, 1H), 7.84 (ddd, $J$ = 0.8, 8.1, 5.2 Hz, 1H), 8.35 (dt, $J$ = 1.8, 8.1 Hz, 1H), 8.75 (dd, $J$ = 1.4, 5.4 Hz, 1H), 8.78 (d, $J$ = 1.5 Hz, 1H). Anal. ($C_{11}H_{13}N_3 \cdot 2.0C_2H_2O_4$) C, H, N.

**(2′*S*,4′*R*)-4′-(Acetyloxy)nicotine Dioxalate (19).** To a sample of 4′-hydroxynicotine (95 mg, 0.53 mmol) (as the borane complex intermediate from compound **13** shown above) in methylene chloride (3 mL) were added acetic anhydride (0.075 mL, 0.80 mmol) and pyridine (0.086 mL, 1.06 mmol), and the solution was allowed to stir at room temperature for 16 h. The solvent was then removed, and the residue was dissolved in ethanol (4 mL). To this was added CsF (184.2 mg, 1.8 mmol), and the mixture was stirred at 57 °C for 16 h. The solvent was removed and the residue purified by chromatography on silica gel, eluting with chloroform/methanol (100:7), to give the product as an oil. This was converted to the dioxalate salt following the procedure described above giving a white solid. Mp: 58–60 °C. $[\alpha]_D$ +6.67° ($c$ 0.24, MeOH). MS (DCI/$NH_3$): $m/z$ 221 $(M + H)^+$, 238 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): $\delta$ 2.18 (s, 3H), 2.7–2.9 (m, 2H), 2.94 (s, 3H), 3.61 (d, $J$ = 14 Hz, 1H), 4.33 (dd, $J$ = 5.5, 14, 1H), 5.04 (q, $J$ = 6.2 Hz, 1H), 5.59 (br t, 1H), 8.05 (dd, $J$ = 5.5, 8.5 Hz, 1H), 8.61 (d, $J$ = 8.5 Hz, 1H), 8.88 (d, $J$ = 5.5 Hz, 1H), 8.97 (s, 1H). Anal. ($C_{12}H_{16}N_2O_2 \cdot 2.2C_2H_2O_4$) C, H; N: calcd, 6.70; found, 6.17.

**(3′*RS*,5′*S*)-3′-(Hydroxymethyl)cotinine (20).** The enolate of cotinine was generated by dropwise addition of a 1.5 M solution of lithium diisopropyl amide solution (1.67 mL, 2.5 mmol) in THF to cotinine (352 mg, 2.00 mmol) in THF (18 mL) at −78 °C. After the mixture was stirred at −78 °C for 15 min, the reaction temperature was raised to 0 °C and the resultant solution allowed to stirred for an additional 30 min. The enolate solution was cooled to −78 °C followed by passage of anhydrous gaseous formaldehyde in a stream of nitrogen (the formaldehyde was generated by the thermal depolymerization of paraformaldehyde at 160 °C). After 2 h at −78 to −20 °C, the reaction was quenched at 0 °C with methanol and the organic solvent was concentrated *in vacuo* to give a dark yellow oil. The oil was purified by flash column chromatography on silica gel, eluting with chloroform/methanol (100:7), to give 242 mg (59% yield) of the title compound as a colorless oil. MS (DCI/$NH_3$): $m/z$ 207 $(M + H)^+$, 224 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.68–1.80 (m, 1H, overlap with water peak), 2.05 (ddd, $J$ = 3.0, 6.0, 9.0 Hz, 1H), 2.23 (m, 1H), 2.41 (dt, $J$ = 12, 9 Hz, 1H), 2.65 (s, 1H), 2.76 (s, 2H), 3.72–3.83 (m, 1H), 3.93–4.05 (m, 1H), 4.52 (t, $J$ = 7.5 Hz, $^1/_3$H), 4.60 (dd, $J$ = 9, 3.0 Hz, $^2/_3$H), 7.47–7.53 (m, $^2/_3$H), 7.59–7.64 (m, $^1/_3$H), 8.47–8.66 (m, 1H), 8.57–8.66 (m, 1H).

**(2′*S*,4′*R*)- and (2′*S*,4′*S*)-4′-(Hydroxymethyl)nicotine (21 and 22).** Compound **20** (735 mg, 3.57 mmol), a mixture of diastereoisomers, in THF (10 mL) was treated dropwise with a 1 M solution of borane (10.7 mL, 10.7 mmol) in THF at room temperature. After the solution was refluxed for 3 h, the reaction was quenched by addition of a large excess of methanol. The resultant solution was allowed to stir at room temperature for an additional 15 min. Solvent was then removed under reduced pressure to give a white solid. One-fifth of this crude reaction product (160 mg, 0.83 mmol) was dissolved in dioxane (8 mL) and treated with cesium fluoride (290 mg, 2.50 mmol) as described in the preparation of compound **13**. The crude product was purified by flash column chromatography on silica gel, eluting with chloroform/methanol (10:1), to give, in order of elution, 23 mg of (2′*S*,4′*S*)-4′-(hydroxymethyl)nicotine (**22**)[26] (14% for two steps) and 69 mg of (2′*S*,4′*R*)-4′-(hydroxymethyl)nicotine (**21**)[26] as a colorless oil.

**4′*S* Isomer.** MS (DCI/$NH_3$): $m/z$ 193 $(M + H)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.65–1.78 (m, 1H), 2.18 (s, 3H), 2.51–2.63 (m, 1H), 3.13–3.30 (m, 2H), 3.66 (dd, $J$ = 4.5, 9.0 Hz, 1H), 3.79 (dd, $J$ = 4.5, 9.0 Hz, 1H), 7.29 (m, 1H, overlap with $CDCl_3$), 7.83 (d, $J$ = 7.5 Hz, 1H), 8.53 (m, 2H). Anal. ($C_{11}H_{16}N_2O \cdot 0.05CHCl_3$) C, H, N.

**4′*R* Isomer.** MS (DCI/$NH_3$): $m/z$ 193 $(M + H)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.88–2.10 (m, 2H), 2.19 (s, 3H), 2.54–2.72 (m, 1H), 3.24 (t, $J$ = 7.5 Hz, 1H), 3.45 (t, $J$ = 8.0 Hz, 1H), 3.66 (m, 2H), 7.28 (m, 1H, overlap with $CDCl_3$), 7.76 (d, $J$ = 7.5 Hz, 1H), 8.52 (dd, $J$ = 3.0, 4.5 Hz, 1H), 8.54 (d, $J$ = 3.0 Hz, 1H). Anal. ($C_{11}H_{16}N_2O$) C, H, N.

**(2′*S*,4′*R*)-4′-[(Methylsulfonyl)oxy]methylnicotine (23).** A sample of 4-(hydroxymethyl)nicotine (**21**) (384 mg, 2.0 mmol) in THF (10 mL) was treated dropwise with a 1 M solution of borane (3.0 mL, 3.0 mmol) in THF at room temperature. After the mixture was refluxed for 3 h, the reaction was quenched by addition of a large excess of methanol. The resultant solution was allowed to stir at room temperature for an additional 15 min. The solvent was then removed under reduced pressure to give a white solid, which was used for next the reaction without further purification. Thus, in a 50 mL round-bottomed flask equipped with a rubber septum and a magnetic stir bar were placed the product (440 mg, 2.0 mmol) from the previous reaction and dichloromethane (25 mL). To this stirred solution was added pyridine (0.34 mL, 8.0 mmol) followed by methanesulfonyl chloride (0.42 mL, 3.0 mmol) at room temperature. The reaction mixture was stirred for 19 h and then the reaction quenched with methanol to destroy excess methanesulfonyl chloride. The reaction mixture was concentrated with a rotary evaporator to obtain a dark yellow oil. This crude material was subjected to flash column chromatography on silica gel, gradually increasing the polarity of the eluent from 2:1 hexane/acetone to 1:1 hexane/acetone, to obtain 350 mg (65% yield) of the borane complex of the methanesulfonate ester as a pale yellow viscous oil. MS (DCI/$NH_3$): $m/z$ 271 $(M + H)^+$, 300 $(M + BNH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.88–1.95 (m, 1H), 2.10–2.20 (m, 1H), 2.32 (s, 1H), 2.50 (s, 2H), 2.78–2.96 (m, 2H), 3.04 (s, 1H), 3.09 (s, 2H), 3.30–3.48 (m, 1H), 3.70–3.88 (m, 1H), 4.25–4.34 (m, 2H), 7.53–7.62 (m, 1H), 8.24 (d, $J$ = 9.0 Hz, $^1/_3$H), 8.40 (d, $J$ = 9 Hz, $^2/_3$H), 8.50–8.72 (m, 2H).

**(2′*S*,4′*R*)-4′-(Fluoromethyl)nicotine Dioxalate (24).** Compound **23** (104 mg, 0.39 mmol) was placed in a 10 mL round-bottomed flask, and 1 M tetra-*n*-butylammonium fluoride (2.31 mL, 2.31 mmol) in THF was added. This solution was heated at reflux for 3 h. The reaction mixture was concentrated, and the residue was taken up in chloroform. The crude material was purified by flash column chromatography (50 g of silica gel), eluting with hexane/acetone (1:2), to provide 12 mg (16% yield) of the title compound as a yellow oil. MS (DCI/$NH_3$): $m/z$ 195 $(M + H)^+$, 212 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 1.92–2.17 (m, 2H), 2.23 (s, 3H), 2.60–2.92 (m, 1H), 3.11–3.47 (m, 1H), 3.40–3.60 (m, 1H), 4.43 (dd, $J$ = 6.0, 48 Hz, 2H), 7.25 (m, 1H, overlap with $CDCl_3$ peak), 7.65–7.90 (m, 1H), 8.55 (m, 2H).

To the solution of the product from above in diethyl ether (1.5 mL) was added oxalic acid in diethyl ether (5 mL) dropwise at 0 °C. After stirring at 0 °C for 15 min, the precipitate was collected by centrifugation, washed with diethyl ether three times, and dried *in vacuo* to yield the title compound as a white powder. Mp: 89–92 °C. $[\alpha]_D$ +9.6° ($c$ 0.12, MeOH). MS (DCI/$NH_3$): $m/z$ 195 $(M + H)^+$, 212 $(M + NH_4)^+$. $^1H$ NMR

($D_2O$): δ 2.52–2.76 (m, 2H), 2.84 (s, 3H), 3.0–3.23 (m, 1H), 3.30–3.43 (m, 1H), 4.01–4.13 (m, 1H), 4.64 (dd, $J$ = 56.0, 48 Hz, 2H), 4.67–4.80 (m, 1H, overlap with $D_2O$ peak), 7.91 (dd, $J$ = 7.5, 4.5 Hz, 1H), 8.45 (d, $J$ = 9.0, 1H), 8.81 (d, $J$ = 6.1 Hz, 1H), 8.87 (s, 1H). Anal. ($C_{11}H_{15}N_2F \cdot 2.1C_2H_2O_4 \cdot 0.5Et_2O$) C, H, N.

**(2′*S*,4′*R*)-4′-(Cyanomethyl)nicotine Dioxalate (25).** A sample of **23** (65 mg, 0.24 mmol) and NaCN (118 mg, 2.4 mmol) were dissolved in DMF (2.5 mL) and water (0.4 mL) and stirred at 100 °C for 16 h. The solvent was removed by evaporation, and the residue was purified on a silica gel column, eluting with 1:1 acetone/hexane. Removal of the solvent gave 32 mg of the free base, which was converted to the oxalate salt as described above. Mp: 55–58 °C. $[\alpha]_D$ +5.33° ($c$ 0.15, MeOH). MS (DCI/$NH_3$): $m/z$ 202 (M + H)$^+$, 219 (M + $NH_4$)$^+$. $^1$H NMR ($D_2O$): δ 2.5–2.6 (m, 1H), 2.7–2.92 (m, 7H), 3.1–3.2 (m, 1H), 3.21–3.41 (m, 1H), 4.12 (m, 1H), 7.85 (dd, $J$ = 8.1, 5.2 Hz, 1H), 8.37 (dt, $J$ = 8, 1.6 Hz, 1H), 8.78 (dd, $J$ = 5.5, 1.5 Hz, 1H), 8.83 (d, $J$ = 1.8 Hz, 1H). Anal. ($C_{12}H_{15}N_3 \cdot 2C_2H_2O_4$) C, N, H.

**(2′*S*,4′*R*)-4′-[(Methylthio)methyl]nicotine Dioxalate (26).** A sample of **23** (260 mg, 1.01 mmol) and NaSMe (175 mg, 2.50 mmol) were dissolved in DMF (4.0 mL) and water (0.4 mL) and stirred at 55 °C for 16 h. The solvent was removed by evaporation, and the residue was purified on a silica gel column, eluting with 20:1 chloroform/methanol. Removal of the solvent gave 65 mg (29%) of the free base, which was converted to the oxalate salt as described above. Mp: 117–120 °C. $[\alpha]_D$ +13° ($c$ 0.10, MeOH). MS (DCI/$NH_3$): $m/z$ 223 (M + H)$^+$, 240 (M + $NH_4$)$^+$. $^1$H NMR ($D_2O$): δ 2.14 (s, 3H), 2.48–2.59 (m, 1H), 2.61–2.72 (m, 1H), 2.78–2.89 (m, 2H), 2.85 (s, 3H), 2.97–3.17 (m, 1H), 3.17–3.34 (m, 1H), 3.60–3.80 (m, 1H), 4.08 (m, 1H), 8.08 (dd, $J$ = 8.1, 5.5 Hz, 1H), 8.64 (m, 1H), 8.88 (d, $J$ = 5.5, 1H), 8.96 (m, 1H). Anal. ($C_{12}H_{18}N_2S \cdot 2.2C_2H_2O_4$) C, H, N.

**(2′*S*,4′*R*)-4′-(Methoxymethyl)nicotine Dioxalate (27).** Compound **20** (195 mg, 0.95 mmol) was dissolved in THF (9 mL) containing sodium hydride (90 mg, 2.25 mmol) and stirred at room temperature for 15 min. Tetrabutylammonium iodide (185 mg, 0.5 mmol) and methyl iodide (0.095 mL, 1.53 mmol) were then added to the reaction mixture. After stirring at room temperature for 20 h, the solvent was evaporated under reduced pressure. The crude product was used directly for the next reaction without further purification. The compound obtained from above was treated with borane followed by cesium fluoride as described in the preparation of compound **13**. The crude product was purified by flash chromatography on silica gel, eluting with acetone/hexane (2:1), to give 24 mg of (2′*S*,4′*R*)-4′-(methoxymethyl)nicotine (12% for three steps). The product from above was converted to the oxalate salt by the procedure described in the preparation of compound **13** in quantitative yield to give 45 mg of the title compound as a very hygroscopic salt. Mp: 102–105 °C. $[\alpha]_D$ +15.9° ($c$ 0.19, MeOH). MS (DCI/$NH_3$): $m/z$ 207 (M + H)$^+$, 224 (M + $NH_4$)$^+$. $^1$H NMR ($D_2O$): δ 1.60–1.70 (m, 1H), 2.41–2.68 (m, 1H), 2.82 (s, 3H), 3.14–3.30 (m, 2H), 3.42 (s, 3H), 3.61 (m, 2H), 4.01 (br s, 1H), 7.97 (dd, $J$ = 5, 8.1 Hz, 1H), 8.49 (m, 1H), 8.82 (d, $J$ = 5.6 Hz, 1H), 8.88 (m, 1H). Anal. ($C_{12}H_{18}N_2O \cdot 2.18C_2H_2O_4 \cdot 0.3H_2O$) C, H, N.

**(3′*RS*,5′*S*)-3′-Methylcotinine (28).** 1.5 M solution of lithium diisopropyl amide solution (3.40 mL, 5.11 mmol) in THF was added dropwise to a solution of cotinine (819 mg, 4.65 mmol) in THF (30 mL) at −78 °C. After the mixture was stirred at −78 °C for 15 min, the reaction temperature was raised to 0 °C and the resultant solution was stirred for an additional 30 min. The solution was cooled to −78 °C, and then methyl iodide (0.304 mL, 4.88 mmol) was added dropwise. After 2 h at −78 to −20 °C, the reaction was quenched at 0 °C with methanol. The organic solvent was concentrated *in vacuo* to give a yellow oil. The oil was purified by flash chromatography on silica gel, eluting with acetone/hexane (3:1), to give 683 mg (77%) of the title compound as a colorless oil. MS (DCI/$NH_3$): $m/z$ 190 (M + H)$^+$, 208 (M + $NH_4$)$^+$. $^1$H NMR ($CDCl_3$): δ 1.27 (d, $J$ = 7.5 Hz, 3H), 2.12–2.20 (m, 2H), 2.63–2.74 (m, 1H), 2.74 (s, 3H), 4.54 (dd, $J$ = 4.5, 8.0 Hz, 1H), 7.33 (ddd, $J$ = 1.0, 4.5, 8.0 Hz, 1H), 7.48 (dt, $J$ = 2.5, 8.0 Hz, 1H), 8.49 (d, $J$ = 2.5 Hz, 1H), 8.59 (dd, $J$ = 2.0, 4.5 Hz, 1H).

**(2′*S*,4′*R*)-4′-Methylnicotine Dioxalate (29).** Compound **28** (40 mg, 0.227 mmol) was treated dropwise with a 1 M solution of borane (0.45 mL, 0.45 mmol) in THF. After 3 h, the reaction was complete; the crude reaction product was treated with cesium fluoride as described in the preparation of the free amine **13**. The crude product was purified by flash column chromatography on silica gel, eluting with acetone/hexane (1:1), to give the title compound (32 mg, 81%) as a colorless oil.[26] MS (DCI/$NH_3$): $m/z$ 177 (M + H)$^+$, 191 (M + $NH_4$)$^+$. $^1$H NMR ($CDCl_3$): δ 1.07 (d, $J$ = 7.0 Hz, 3H), 1.78–1.83 (m, 1H), 1.93–2.0 (m, 2H), 2.16 (s, 3H), 2.42–2.50 (m, 1H), 3.21 (t, $J$ = 8.1 Hz, 1H), 3.36 (dd, $J$ = 7, 9.2 Hz, 1H), 7.26 (dd, $J$ = 4.5, 8 Hz, 1H), 7.70 (dt, $J$ = 2, 8 Hz, 1H), 8.49 (dd, $J$ = 2, 4.5 Hz, 1H), 8.52 (d, $J$ = 2 Hz, 1H).

To a solution of the product obtained above in diethyl ether (1.5 mL) was added oxalic acid in diethyl ether dropwise at 0 °C. The solution was stirred at 0 °C for 15 min, and the precipitate was collected by centrifugation, washed with diethyl ether three times, and dried *in vacuo* to yield the title compound as a white powder. Mp: 112–115 °C. $[\alpha]_D$ +16.3° ($c$ 0.14, MeOH). MS (DCI/$NH_3$): $m/z$ 177 (M + H)$^+$, 194 (M + $NH_4$)$^+$. $^1$H NMR ($D_2O$): δ 1.23 (d, $J$ = 6.6 Hz, 3H), 2.28–2.42 (m, 1H), 2.54–2.68 (m, 1H), 2.80 (m, 1H, overlap with 2.84 peak), 2.84 (s, 3H), 3.04 (m, 1H), 4.72 (br s, 1H), 3.98 (m, 1H), 7.98 (dd, $J$ = 5.5, 8.0 Hz, 1H), 8.49 (d, $J$ = 8.1, 1 Hz, 1H), 8.83 (d, $J$ = 5.5 Hz, 1H), 8.89 (s, 1H). Anal. ($C_{11}H_{16}N_2 \cdot 2.4C_2H_2O_4 \cdot H_2O$) C, H, N.

**(2′*S*,4′*R*)-4′-Ethylnicotine Dioxalate (31).** Following a similar procedure as described for **28**, cotinine (300 mg, 1.70 mmol) was reacted with 1.5 mL of LDA (1.36 mL, 2.0 mmol) followed by treatment with ethyl iodide (0.204 mL, 2.55 mmol) to give 240 mg (69% yield) of 3′-ethylcotinine (**30**) as a colorless oil. MS (DCI/$NH_3$): $m/z$ 205 (M + H)$^+$, 222 (M + $NH_4$)$^+$. $^1$H NMR ($CDCl_3$): δ 0.98 (t, $J$ = 7.5 Hz, 3H), 1.50 (m, 1H), 1.94 (m, 1H), 2.23 (m, 1H), 2.58 (m, 1H), 2.73 (s, 3H), 4.54 (dd, $J$ = 3, 9 Hz, 1H), 7.35 (m, 1H), 7.50 (m, 1H), 8.49 (br s, 1H), 8.60 (br s, 1H).

Following a similar procedure as described for **29**, the product (240 mg, 1.26 mmol) obtained from above was treated with borane (3.79 mL, 3.79 mmol) followed by decomplexation with cesium fluoride (292 mg, 2.52 mmol) to afford 136 mg of the title compound (56% yield for two steps) as a colorless oil. MS (DCI/$NH_3$): $m/z$ 191 (M + H)$^+$, 208 (M + $NH_4$)$^+$. $^1$H NMR ($CDCl_3$): δ 0.92 (t, $J$ = 7.5 Hz, 3H), 1.36 (dd, $J$ = 7.5, 13.5 Hz, 1H), 1.44 (dd, $J$ = 7.5, 13.5 Hz, 1H), 1.78–1.93 (m, 1H), 1.93–2.06 (m, 1H), 2.16 (s, 3H), 2.16–2.37 (m, 1H), 3.26 (m, 1H), 3.37 (m, 1H), 7.25 (m, 1H, overlap with $CHCl_3$), 7.71 (m, 1H), 8.49 (dd, $J$ = 3.0, 6.0 Hz, 1H), 8.52 (d, $J$ = 3 Hz, 1H).

The product obtained above was converted to the dioxalate salt following the procedure described in the preparation of **29**. Mp: 65–67 °C. $[\alpha]_D$ +12° ($c$ 0.11, MeOH). MS (DCI/$NH_3$): $m/z$ 177 (M + H)$^+$, 194 (M + $NH_4$)$^+$. $^1$H NMR ($D_2O$): δ 0.97 (t, $J$ = 7.5 Hz, 3H), 1.61 (m, 2H), 2.31–2.46 (m, 1H), 2.50–2.70 (m, 2H), 2.82 (s, 3H), 3.04–3.14 (m, 1H), 3.90–4.04 (m, 1H), 4.60–4.70 (m, 1H, overlap with $D_2O$ peak), 7.79 (dd, $J$ = 4.5, 8.0 Hz, 1H), 8.29 (d, $J$ = 9.0, 1.5 Hz, 1H), 8.74 (d, $J$ = 6.08 Hz, 1H), 8.78 (d, $J$ = 1.5 Hz, 1H). Anal. ($C_{12}H_{18}N_2 \cdot C_2H_2O_4$) C, H, N.

**(3′*R*,5′*S*)-3′-Benzylcotinine (32).** Following a similar procedure as described for **28**, cotinine (300 mg, 1.70 mmol) was reacted with 1.5 M of LDA (1.48 mL, 2.22 mmol), followed by treatment with benzyl bromide (0.303 mL, 2.25 mmol) to give 410 mg (90%) of the title compound as a colorless oil. MS (DCI/$NH_3$): $m/z$ 267 (M + H)$^+$, 284 (M + $NH_4$)$^+$. $^1$H NMR ($CDCl_3$): δ 1.88–1.98 (m, 1H), 2.25–2.37 (m, 1H), 2.74 (s, 3H), 2.81 (dd, $J$ = 8.0, 13.5 Hz, 1H), 2.90–3.01 (m, 1H), 3.22 (dd, $J$ = 4.0, 13.5 Hz, 1H), 4.21 (dd, $J$ = 4.0, 8.0 Hz, 1H), 7.16–7.43 (m, 1H), 7.52 (d, $J$ = 8.0 Hz, 1H), 8.41 (m, 1H), 8.57 (d, $J$ = 4.5 Hz, 1H).

**(2′*S*,4′*R*)-4′-Benzylnicotine Dioxalate (33).** Following a similar procedure as described for **29**, the product **32** (410 mg, 1.54 mmol) obtained from above was treated with borane (4.62 mL, 4.62 mmol) followed by decomplexation with cesium fluoride (535 mg, 4.62 mmol) to afford the crude product. This

material was purified by flash column chromatography on silica gel, eluting with chloroform/methanol (10:1), to give 120 mg of the title compound (31% for two steps) as a colorless oil. MS (DCI/$NH_3$): *m/z* 253 $(M + H)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.53 (d, $J$ = 2.2 Hz, 1H), 8.50 (d, $J$ = 4.4 Hz, 1H), 7.6–7.7 (m, 1H), 7.10–7.31 (m, 6H), 3,16–3.45 (m, 2H), 2.73 (s, 3H), 2.19–2.33 (m, 4H), 1.92–2.19 (m, 2H).

To a solution of the product obtained above in diethyl ether (1.5 mL) was added oxalic acid in diethyl ether dropwise at 0 °C. After stirring at 0 °C for 15 min, the precipitate was collected by centrifugation, washed with diethyl ether (3×), and dried *in vacuo* to yield the title compound as a white powder. Mp: 63–65 °C. $[\alpha]_D$ +13.8° (*c* 0.17, MeOH). MS (DCI/$NH_3$): *m/z* 253 $(M + H)^+$, 270 $(M + NH_4)^+$. $^1H$ NMR ($D_2O$): $\delta$ 2.50–2.52 (m, 2H), 2.82 (s, 3H), 2.94 (d, $J$ = 8.1 Hz, 1H), 3.01–3.24 (m, 1H), 3.14–3.27 (m, 1H), 3.82–3.96 (m, 1H), 4.6–4.83 (m, 1H, overlap with $D_2O$ peak), 7.32–7.42 (m, 5H), 7.93 (dd, $J$ = 5.5, 8.4 Hz, 1H), 8.43 (m, 1H), 8.79 (dd, $J$ = 1.5, 5.6 Hz, 1H), 8.84 (d, $J$ = 2.9 Hz, 1H). Anal. ($C_{17}H_{20}N_2 \cdot C_2H_2O_4$) C, H, N.

**5′-Hydroxy-5′-methylnicotine (34).** A sample of cotinine (0.95 g, 5.4 mmol; from Aldrich Chemical Co.) was dissolved in anhydrous ether (25 mL) flushed with nitrogen. The solution was cooled to 0 °C, and methyllithium (4.70 mL, 6.6 mmol) was slowly added via a syringe. A white precipitate formed, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with 1 M HCl (10 mL), and then potassium carbonate (0.7 g) was added. The layers were separated, the organic layer was removed, and the residue was dissolved in ethyl acetate. The aqueous layer was extracted overnight with ethyl acetate, and the two fractions were combined and then dried over sodium sulfate and concentrated to afford 1.11 g of a clear orange oil, which was purified on silica gel, eluting with chloroform/methanol (20:1) containing 1% ammonium hydroxide increasing to 7:1 containing 2% ammonium hydroxide. Removal of the solvent gave 0.27 g of the intermediate product.

**(2′*S*,5′*S*)-5′-Methylnicotine Oxalate (36).** Compound **34** (0.27 g, 1.4 mmol) was dissolved in anhydrous methanol (5.6 mL) under an nitrogen atmosphere and adjusted to the bromocresol green acidic end point (yellow) with 2 M HCl in anhydrous methanol. To this was added sodium cyanoborohydride (88 mg, 1.4 mmol), and the pH was again adjusted to acidic with the HCl. The reaction mixture was stirred for 0.5 h and the reaction quenched with 0.1 M sodium hydroxide (6 mL). The solution was adjusted to pH 12 with 15% sodium hydroxide solution; then solid sodium chloride and brine were added. The mixture was extracted with ethyl acetate, dried, and purified on silica gel, eluting with a series of increasingly polar mixtures of chloroform/methanol containing a small amount of ammonium hydroxide. Two fractions were isolated. Fraction A consisted of 95 mg (38%) of the 5′-(*S*)-methyl isomer. $[\alpha]_{546}$ −95.4° (*c* 0.5, MeOH) (lit.[28] $[\alpha]_{546}$ −96.4° (*c* 0.6, MeOH). MS (DCI/$NH_3$): *m/z* 177 $(M + H)^+$, 194 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.54 (d, $J$ = 2.5 Hz, 1H), 8.49 (dd, $J$ = 2.0, 6.0 Hz, 1H), 7.73 (m, 1H), 7.25 (dd, $J$ = 3.0, 7.5 Hz), 3.22 (t, $J$ = 8 Hz, 1H), 2.33–2.46 (m, 1H), 2.11 (s, 3H), 1.91–2.04 (m, 1H), 1.47–1.73 (m, 2H), 1.21 (d, $J$ = 6.0 Hz, 3H). Fraction B consisted of 34 mg (14%) of the 5′-(*R*)-methyl isomer. $[\alpha]_{546}$ −66.1° (*c* 0.5, MeOH) (lit.[28] $[\alpha]_{546}$ −65.2° (*c* 0.23, MeOH). MS (DCI/$NH_3$): *m/z* 177 $(M + H)^+$, 194 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.51 (d, $J$ = 2.5 Hz, 1H), 8.49 (dd, $J$ = 2.0, 6.0 Hz, 1H), 7.64 (dt, $J$ = 3, 7.5 Hz, 1H), 7.25 (dd, $J$ = 3.0, 7.5 Hz), 3.71 (dd, $J$ = 6.0, 9.0 Hz, 1H), 3.49 (m, 1H), 2.17–2.42 (m, 2H), 2.15 (s, 3H), 1.67–1.81 (m, 1H), 1.51–1.62 (m, 1H), 1.05 (d, $J$ = 6.0 Hz, 3H). Each fraction was converted to the oxalate salt following the procedure described before.

**Fracti n A (5′*S* Isomer).** Mp: 93–96 °C. $[\alpha]_D$ +30.3° (*c* 0.29, MeOH). MS (DCI/$NH_3$): *m/z* 177 $(M + H)^+$, 194 $(M + NH_4)^+$. $^1H$ NMR ($CD_3OD$): $\delta$ 1.53 (d, 3H), 2.05 (m, 1H), 2.35–2.6 (m, 4H), 2.76 (s, 3H), 3.62 (br m, 1H), 4.53 (t, 1H), 7.60 (q, 1H), 8.14 (dt, $J$ = 1.5, 7.5 Hz, 1H), 8.67 (dd, $J$ = 2.5, 6 Hz, 1H), 8.73 (d, $J$ = 2.5 Hz, 1H). Anal. ($C_{11}H_{16}N_2 \cdot 1.3C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**(2′*S*,5′*R*)-5′-Methylnicotine Oxalate (35). Fraction B (5′*R* Isomer).** Viscous oil. MS (DCI/$NH_3$): *m/z* 177 $(M + H)^+$, 194 $(M + NH_4)^+$. $^1H$ NMR ($CD_3OD$): $\delta$ 1.47 (d, 3H), 1.93–2.07 (m, 1H), 2.4–2.6 (m, 4H), 2.58 (s, 3H), 3.95 (br s, 1H), 7.59 (q, 1H), 8.10 (dt, $J$ = 1.5, 7 Hz, 1H), 8.67 (dd, $J$ = 2.5, 6 Hz, 1H), 8.73 (d, $J$ = 2.5 Hz, 1H). Anal. ($C_{11}H_{16}N_2 \cdot 1.5C_2H_2O_4 \cdot 0.4H_2O$) C, H, N.

**(2′*S*,5′*S*)-5′-Butylnicotine Oxalate (38).** Following a similar procedure as described above, cotinine (1.00 g, 5.67 mmol) was reacted with *n*-butyllithium (2.70 mL, 6.75 mmol) to afford 1.33 g of the amino alcohol as an oil. Following a similar procedure as described above, the aminal product was reduced with sodium cyanoborohydride. Chromatography provided two fractions; their physical data are shown below. Fraction A (5′*S* isomer) consisted of 360 mg (37%). $[\alpha]_D$ −36.2° (*c* 0.5, MeOH) (lit.[28] $[\alpha]_D$ −37.6° (*c* 1.9, MeOH)). MS (DCI/$NH_3$): *m/z* 219 $(M + H)^+$, 236 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.53 (d, $J$ = 2.5 Hz, 1H), 8.43 (dd, $J$ = 2.0, 6.0 Hz, 1H), 7.71 (dt, $J$ = 3.0, 7.5 Hz, 1H), 7.24 (dd, $J$ = 3.0, 7.5 Hz), 3.23 (t, $J$ = 8 Hz, 1H), 2.26–2.38 (m, 1H), 2.11 (s, 3H), 1.91–2.15 (m, 2H), 1.50–1.78 (m, 3H), 1.34–1.44 (m, 4H), 0.93 (t, $J$ = 7.5 Hz, 3H). Fraction B consisted of 77 mg (8%) of the 5′-(*R*)-methyl isomer. $[\alpha]_D$ −86.7° (*c* 0.5, MeOH) (lit.[28] $[\alpha]_D$ −85.1° (*c* 0.1, MeOH)). MS (DCI/$NH_3$): *m/z* 219 $(M + H)^+$, 236 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.48 (m, 1H), 7.61 (dt, $J$ = 3, 7.5 Hz, 1H), 7.25 (dd, $J$ = 3.0, 7.5 Hz), 3.79 (dd, $J$ = 6.0, 7.5 Hz, 1H), 3.02–3.13 (m, 1H), 2.22–2.39 (m, 1H), 2.07–2.20 (m, 1H), 2.16 (s, 3H), 1.60–1.83 (m, 2H), 1.15–1.45 (m, 4H), 0.94 (t, $J$ = 7.5 Hz, 3H). Each fraction was converted to the oxalate salt as described above. The physical data of each salt are shown below.

**Fraction A (5′*S* Isomer).** Mp: 67–70 °C. $[\alpha]_D$ +51.6° (*c* 0.18, MeOH). MS (DCI/$NH_3$): *m/z* 219 $(M + H)^+$, 236 $(M + NH_4)^+$, 437 $(2M + H)^+$. $^1H$ NMR ($CD_3OD$): $\delta$ 0.96 (m, 3H), 1.35–1.53 (m, 4H), 1.63–1.76 (m, 1H), 1.95–2.13 (m, 2H), 2.35–2.65 (m, 3H), 2.75 (s, 3H), 3.48 (br q, 1H), 4.49 (br t, 1H), 7.58 (q, 1H), 8.14 (dt, $J$ = 1.5, 7.5 Hz, 1H), 8.67 (dd, $J$ = 2.5, 6 Hz, 1H), 8.74 (d, $J$ = 2.5 Hz, 1H). Anal. ($C_{14}H_{22}N_2 \cdot 1.4C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**(2′*S*,5′*R*)-5′-Butylnicotine Oxalate (37). Fraction B (5′*R* Isomer).** Viscous oil. $[\alpha]_D$ −4.68° (*c* 0.16, MeOH). MS (DCI/$NH_3$): *m/z* 219 $(M + H)^+$, 236 $(M + NH_4)^+$. $^1H$ NMR ($CD_3OD$): $\delta$ 0.97 (m, 3H), 1.35–1.53 (m, 4H), 1.64–1.76 (m, 1H), 1.90–2.05 (m, 2H), 2.45–2.6 (m, 7H), 3.70 (br s, 1H), 7.58 (q, 1H), 8.07 (dt, $J$ = 1.5, 7.5 Hz, 1H), 8.67 (dd, $J$ = 2.5, 6 Hz, 1H), 8.72 (d, $J$ = 2.5 Hz, 1H). Anal. ($C_{14}H_{22}N_2 \cdot 1.4C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**(2′*S*,5′*R*)-5′-Phenylnicotine Oxalate (40).** Following a similar procedure as described above, cotinine (1.00 g, 5.67 mmol) was reacted with phenyllithium (4.25 mL, 8.50 mmol) to afford 1.33 g of the amino alcohol compound as an oil. Following a similar procedure as described above, the phenyl amino alcohol was reduced with sodium cyanoborohydride. Chromatography provided two fractions; their physical data are shown below. Fraction A (5′*R* isomer) consisted of 572 mg (40%). $[\alpha]_D$ −8.72° (*c* 0.5, MeOH) (lit.[28] $[\alpha]_D$ −8.3° (*c* 6.1, MeOH)). MS (DCI/$NH_3$): *m/z* 238 $(M + H)^+$, 256 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.66 (d, $J$ = 2.5 Hz, 1H), 8.52 (dd, $J$ = 2.0, 6.0 Hz, 1H), 7.84 (dt, $J$ = 3.0, 7.5 Hz, 1H), 7.27 (dd, $J$ = 3.0, 7.5 Hz), 7.27–7.49 (m, 5H), 3.46 (m, 2H), 2.21–2.30 (m, 2H), 2.03 (s, 3H), 1.76–1.90 (m, 2H). Fraction B consisted of 77 mg (8%) of the 5′-(*S*)-phenyl isomer. $[\alpha]_D$ −89.2° (*c* 0.5, MeOH) (lit.[28] $[\alpha]_D$ −88.9° (*c* 1.26, MeOH)). MS (DCI/$NH_3$): *m/z* 238 $(M + H)^+$, 256 $(M + NH_4)^+$. $^1H$ NMR ($CDCl_3$): $\delta$ 8.55 (d, $J$ = 2.5 Hz, 1H), 8.52 (dd, $J$ = 2.0, 6.0 Hz, 1H), 7.84 (dt, $J$ = 3.0, 7.5 Hz, 1H), 7.27 (dd, $J$ = 3.0, 7.5 Hz), 7.27–7.49 (m, 5H), 3.46 (m, 2H), 2.21–2.30 (m, 2H), 2.03 (s, 3H), 1.76–1.90 (m, 2H). Each fraction was converted to the oxalate salt as described above. The physical data of each salt are shown below.

**Fracti n A (5′*R* Isomer).** Mp: 105–107 °C. $[\alpha]_D$ −43.5° (*c* 0.14, MeOH). MS (DCI/$NH_3$): *m/z* 238 $(M + H)^+$, 256 $(M + NH_4)^+$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.95 (s, 3H), 2.04–2.20 (m, 2H), 2.52–2.63 (m, 2H, overlap with DMSO), 4.31–4.51 (m, 2H), 7.33–7.48 (m, 1H), 7.89 (dd, $J$ = 5.2, 1.5 Hz, 1H), 8.55

(dd, $J$ = 5.2, 1.5 Hz, 1H), 8.64 (m, 1H). Anal. ($C_{16}H_{18}N_2 \cdot 1.9C_2H_2O_4$) C, H, N.

**(2′S,5′S)-5′-Phenylnicotine Oxalate (39). Fraction B (5′S Isomer).** Mp: 115–117 °C. $[\alpha]_D$ −3.33° (c 0.15, MeOH). MS (DCI/$NH_3$): $m/z$ 238 $(M + H)^+$, 256 $(M + NH_4)^+$. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.76–1.98 (m, 2H), 1.97 (s, 3H), 2.20–2.32 (m, 2H), 3.50–3.68 (m, 2H), 7.25–7.52 (m, 1H), 7.93 (dd, $J$ = 5.2, 1.5 Hz, 1H), 8.5 (m, 1H), 8.65 (m, 1H). Anal. ($C_{16}H_{18}N_2 \cdot 2C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**(±)-3′,4′-Dimethylcotinine (41).** A sample of (±)-*trans*-4-methylcotinine (7) (124 mg, 0.65 mmol) was dissolved in dry THF (8 mL), and LDA (0.52 mL, 0.78 mmol) was added. The reaction mixture was stirred at −78 °C for 15 min and at 0 °C for 30 min. The temperature was again lowered to −78 °C, methyl iodide (0.045 mL, 0.72 mmol) was added, and the solution was stirred for 1.5 h. The reaction was quenched at 0 °C with methanol, the organic solvent was concentrated *in vacuo*, and the residue was purified by flash column chromatography on silica gel, eluting with acetone/hexane (1:1), to give 69 mg of the title product.

**(±)-3′,4′-Dimethylnicotine Dioxalate (42).** Product 41 was dissolved in THF (3 mL), and a 1 M solution of borane (1.0 mL, 1.0 mmol) in THF was added. The reaction was refluxed for 3 h. The reaction was quenched by stirring with methanol for 10 min, and the solvent was removed. The residue was treated with cesium fluoride (118 mg) in ethanol (4 mL) as described above. The crude product was purified by flash column chromatography on silica gel, eluting with acetone/hexane (3:4), to give 36 mg of the title compound as a colorless oil. The product was converted to the oxalate salt following the procedure described in the preparation of salt 13. MS (DCI/$NH_3$): $m/z$ 191 $(M + H)^+$, 208 $(M + NH_4)^+$. $^1$H NMR ($D_2O$): $\delta$ 0.95 (d, $J$ = 6.6 Hz, 3H), 1.13 (d, $J$ = 6.9 Hz, 3H), 2.78–2.90 (m, 2H), 2.81 (br s, 3H), 3.05 (br q, 1H), 4.16 (br m, 1H), 4.28 (br m, 1H), 7.90 (m, 1H), 8.40 (dt, $J$ = 6, 2 Hz, 1H), 8.82 (m, 2H). Anal. ($C_{12}H_{18}N_2O_7 \cdot 2C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

**Binding Experiments.** Binding of [$^3$H]cytisine to nicotinic acetylcholine receptors was determined using a modification of the method of Pabreza and co-workers.[33] Membrane-enriched fractions were prepared from whole rat brain following a published method.[34] The tissue was stored as pellets at −80 °C. Prior to use, pellets were slowly thawed, washed, and resuspended in binding buffer [BSS (basic salt solution); 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 50 mM Tris-Cl, pH 7.4, 4 °C]. Samples containing approximately 100 mg of protein were incubated at 4 °C for 75 min with 1.25 nM [$^3$H]cytisine. For concentration–inhibition studies, seven log dilutions of test compounds in duplicate were used. Three separate determinations were done for each compound. Nonspecific binding was determined in the presence of 10 mM (−)-nicotine. Bound radioactivity was isolated by vacuum filtration onto no. 32 glass fiber filters (S&S). The filters were prerinsed with 0.5% poly(ethylenimine) (PEI) prior to sample filtration and rapidly rinsed with 10 mL of ice cold BSS. Filters were counted in 3 mL of Ecolume (ICN). $IC_{50}$ values were calculated with a four-parameter logistics program in RS/1 (BBN), and $K_i$ values were determined using the Cheng–Prusoff equation[35] as shown below.

$$K_i = IC_{50}/(1 + [\text{ligand}]/K_D)$$

**Acknowledgment.** The authors thank Drs. Richard Elliott and David Garvey for useful discussion and Patricia Pavlik for carrying out steric volume and molecular mechanics calculations.

## References

(1) Schoenberg, B. S.; Kokman, E.; Okazaki, H. Alzheimer disease and other dementing illnesses in a defined United States population: incidence rates and clinical features. *Ann. Neurol.* **1987**, *22*, 724–729.
(2) Norberg, A.; Adem, A.; Hardy, J.; Winblad, B. Change in Nicotinic Receptor Subtypes in Temporal Cortex of Alzheimer's Brains. *Neurosci. Lett.* **1986**, *86*, 317–321.
(3) Reisine, T. D.; Yamamura, H. I.; Bird, E. D.; Spokes, E.; Enna, S. J. Pre- and postsynaptic neurochemical alternations in Alzheimer's disease. *Brain Res.* **1978**, *159*, 477–481.
(4) Coyle, J. T.; Price, D. L.; Delong, M. R. Alzheimer's disease: A Disorder of Cortical Cholinergic Innovation. *Science* **1983**, *219*, 1184–1190.
(5) McGeer, P. L.; McGeer, E. G.; Suzuki, J.; Dolman, G. E.; Nagai, T. Aging, Alzheimer's Disease, and The Cholinergic System of The Basal Forebrain. *Neurology* **1984**, *34*, 741–745.
(6) Perry, E. K. The Cholinergic Hypothesis- Ten Years On. *Br. Med. Bull.* **1986**, *42*, 63–69.
(7) Perry, E. K.; Blessed, G.; Tomlinson, B. E.; Perry, R. H.; Crow, T. J.; Cross, A. J.; Dockray, G. J.; Dimaline, R.; Arredui, R. Neurochemical Activities in Human Temporal Lobe Related to Aging and Alzheimer-Type Changes. *Neurobiol. Aging* **1981**, *2*, 251–256.
(8) Kellar, K.; Whitehouse, P. J.; Matino-Barrow, A. M.; Marcus, K.; Price, D. L. Muscarinic and Nicotinic Cholinergic Binding Sites in Alzheimer's Disease Cerebral Cortex. *Brain Res.* **1987**, *436*, 62–68.
(9) Whitehouse, P. J.; Martino, A. M.; Wagster, M. V.; Price, D. L.; Mayeux, R.; Atack, J. R.; Kellar, K. J. Reduction in [$^3$H]Nicotine Acetylcholine Binding in Alzheimer's Disease and Parkinson Disease. *Neurology* **1988**, *38*, 720–723.
(10) For review, see: Giacobini, E. Cholinergic Receptors in Human Brain: Effects of Aging and Alzheimer Disease. *J. Neurosci. Res.* **1990**, *27*, 548–560.
(11) Drachman, D. A.; Leavitt, J. Human Memory and the Cholinergic System. *Arch. Neurol.* **1974**, *30*, 113–121.
(12) Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The Cholinergic Hypothesis of Geriatric Memory Dysfunction. *Science* **1982**, *217*, 408–417.
(13) Prohovnik, I. Muscarinic and Nicotinic Contributions To The AD Cortical Perfusion Abnormality. *Neurobiol. Aging* **1990**, *11*, 262.
(14) Sahakian, B.; Jones, G.; Levy, R.; Gray, J.; Warburton, D. The Effects of Nicotine on Attention, Information Processing, and Short-Term, Memory in Patients with Dementia of Alzheimer Type. *Br. J. Psychiatry* **1989**, *154*, 797–800.
(15) Newhouse, P. A.; Sunderland, Y.; Tariot, P. N.; Blumhardt, C. L.; Weingartner, H.; Mellow, A. Intravenous Nicotine in Alzheimer's Disease: A Pilot Study. *Psychopharmacology* **1988**, *95*, 171–175.
(16) Cregan, E.; Ordy, J. M.; Palmer, E.; Blosser, J.; Wengenack, T.; Thomas, G. Spatial Working Memory Enhancement by Nicotine of Aged Long Evans rats in The T-Maze. *Soc. Neurosci. Abstr.* **1989**, *15*, 2952.
(17) Jason, A. M.; Fuxe, K.; Agnati, L. F.; Jasson, A.; Bjeklke, B.; Sundstrom, E.; Anderson, K.; Harfstrand, A.; Goldstein, M.; Owman, C. Protective Effects of Chronic Nicotine Treatment on Lesioned Nigrostriatal Dopamine Neurons in The Male Rat. *Prog. Brain Res.* **1989**, *79*, 257–265.
(18) Owman, C.; Fuxe, K.; Jason, A. M.; Kahrstrom, J. Studies of Protective Actions of Nicotine on Neuronal and Vascular Functions in the Brain of Rats: Comparison Between Sympathetic Noradrenergic and Mesostriatal Dopaminergic Fiber System, and The Effect of A Dopamine Agonist. *Prog. Brain Res.* **1989**, *79*, 267–276.
(19) Benwall, M. E. M.; Balfour, D. J. K.; Anderson, J. M. Evidence that Tobacco Smoking Increase the Density of (-)-[3H]Nicotine Binding Site in Human Brain. *J. Neurochem.* **1988**, *50*, 1243–1247.
(20) For limited studies, see: Abood, L. G.; Lerner-Marmarosh, N.; Wang, D.; Saraswati, M. Structure-Activity Relationship of Various Nicotinoids and *N*-Substituted Carbamate Esters of Choline and Other Amino Alcohols. *Med. Chem. Res.* **1993**, *2*, 552–563. Glennon, R. A.; Maarouf, A.; Fahmy, S.; Martin, B.; Fan, F.; Yousif, M.; Shafik, R. M.; Dukat, M. Structure-affinity relationships of simple nicotine analogs. *Med. Chem. Res.* **1993**, *2*, 546–551.
(21) Both (±)-*trans*-4-cotininecarboxylic acid and (*S*)-cotinine were obtained from Aldrich Chemical Co.
(22) Cushman, M.; Castagnoli, N., Jr. The Synthesis of *trans*-3′-Methylnicotine. *J. Org. Chem.* **1972**, 37, 1268–1271.
(23) Schummer, D.; Hofle, G. Tris(trimethylsilyl)silane as A Reagent for the Radical Deoxygenation of Alcohols. *Synlett* **1990**, *11*, 705–706.
(24) Jacob, P., III; Shulgin, A. T.; Benowitz, N. L. Synthesis Of (3′*R*,5′*S*)-*trans*-3′- Hydroxycotinine, A Major Metabolite of Nicotine. Metabolic Formation of 3′- Hydroxycotinine in Humans Is Highly Stereoselective. *J. Med. Chem.* **1990**, *33*, 1888–1891.
(25) The stereochemistry of compound 18 was determined by NOESY and DQCOSY studies. H2′ ($\beta$ configuration) was first identified by using DQCOSY. A NOE was observed between H3a′ and H2′. Therefore, the stereochemistry of H3a′ is $\beta$. We have also seen an NOE between H3a′ and H4′. Thus, the stereochemistry of H4′ is assigned as $\beta$. On the basis of this analysis, the configuration of CN should be $\alpha$.
(26) Edwards, W. B., III. Optically Active Nicotine Analogs And Process For Their Preparation. U.S. Patent. 4,332,945, 1982.

(27) Although a very small amount of diastereoisomers was detected on NMR spectra using methyl iodide and ethyl iodide as reagents in alkylation, we failed to isolate the minor isomers due to scarce quantity of material. Surprisingly, none of the minor diastereoisomer was observed with the benzyl analog. The stereochemistry of **29** was determined by NOE and comparing its NMR spectrum with that reported in the literature.[26]
(28) Schibagaki, M.; Matushita, H.; Kaneko, H. The Synthesis of 5'-Alkylnicotines. *Heterocycles* **1986**, *24*, 423–428.
(29) Seeman, J. Recent Studies in Nicotine Chemistry. Conformational Analysis, Chemical Reactivity Studies, and Theoretical Modeling. *Heterocycles* **1984**, *22*, 165–192.
(30) A conformational search was accomplished by using both distance geometry and MMP2. We have found that the lowest energy conformation for both α and β isomers is when the *N*-methyl group is parallel to the plane defined by the C2', C3', C4', and C5' atoms of the pyrrolidine ring. In the α isomer it possesses the energy value of 20.73 kcal/mol, whereas it is 22.72 kcal/mol in the β isomer. Two higher energy conformations have also been identified for the β-methyl isomer. One conformation (*syn*-N-Me, C5'-Me) possesses energy of 2.24 kcal/mol higher than the lowest energy conformation, whereas the second conformation is 2.89 kcal/mol higher than the lowest energy conformation (*anti*-N-Me, C5'-Me). Likewise, the α-methyl isomer has two higher energy conformations. The energy of one conformation (*syn*-N-Me, C5'-Me) is 5.73 kcal/mol higher than the lowest energy conformation, whereas the second conformation is 4.01 kcal/mol higher than the lowest conformation (*anti*-N-Me, C5'-Me).
(31) Gund, T. M.; Spivak, C. E. Pharmacophore for Nicotinic Agonists. *Methods Enzymol.* **1991**, 677–693.
(32) Kosower, E. M. A structural and Dynamic Model for the Nicotinic Acetylcholine Receptor. *Prog. Clin. Biol. Res.* **1989**, *289*, 279–292.
(33) Pabreza, L. A.; Dhawan, S.; Kellar, K. J. [$^3$H] Cytisine Binding to Nicotine Cholinergic Receptors in Brain. *Mol. Pharmacol.* **1991**, *39*, 9–12.
(34) Enna, S. J.; Snyder, S. H. Properties of γ-Aminobutyric Acid Receptor Binding in Rat Brain Synaptic Membrane Fractions. *Brain Res.* **1975**, *100*, 81–87.
(35) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (IC 50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3918.

**PCT** WORLD INTELLECTUAL PRO
International

INTERNATIONAL APPLICATION PUBLISHED UNDE

WO 9608468A1

(51) International Patent Classification 6 :
C07C 271/12, C07D 207/08, 211/22, 295/088, A61K 31/27, 31/40, 31/495

A1

(11) I

(43) International Publication Date: 21 March 1996 (21.03.96)

(21) International Application Number: PCT/DK95/00368

(22) International Filing Date: 14 September 1995 (14.09.95)

(30) Priority Data:
1056/94 14 September 1994 (14.09.94) DK

(71) Applicant *(for all designated States except US)*: H. LUNDBECK A/S [DK/DK]; Ottiliavej 9, DK-2500 Valby Copenhagen (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FALCH, Erik [DK/DK]; Olgasvej 31, DK-2950 Vedbæk (DK). MIKKELSEN, Ivan [DK/DK]; Sankt Nikolaj Vej, 5 D, 1.th., DK-1953 Frederiksberg C (DK). KROGSGAARD-LARSEN, Povl [DK/DK]; Elmevej 25, DK-3450 Allerød (DK).

(74) Agent: MEIDAHL PETERSEN, John; H. Lundbeck A/S, Patent Dept., Ottiliavej 9, DK-2500 Valby Copenhagen (DK).

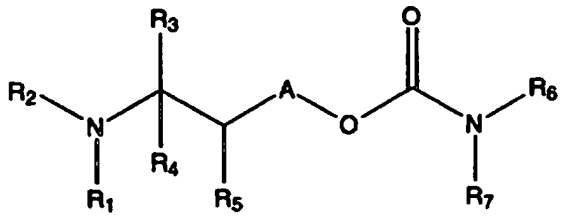
(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

**Published**
*With international search report.*

(54) Title: CARBAMOYLOXY AMINE COMPOUNDS

(57) Abstract

Carbamoyloxypropylamine or carbamoyloxyethylamine compounds of formula (I), wherein A represents $CH_2$ or a bond, $R_1$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or phenyl; and $R_2$ is alkyl, alkenyl, alkynyl, cycloalkyl or phenyl; or $R_1$ and $R_2$ together form a ring; $R_3$ and $R_4$ are hydrogen, alkyl, alkenyl, alkynyl, halogenated alkyl, cycloalkyl, phenyl, or phenylalkyl or $R_3$ and $R_4$ together form a spirojoined $C_{4-7}$ carbocycle; or when $R_1$ and $R_2$ are not linked, $R_3$ and $R_2$ may form ring; $R_5$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, phenyl, or phenylalkyl or together with $R_2$ form a ring; or $R_5$ together with $R_4$ form a ring; $R_6$ and $R_7$ are hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, phenyl or phenylalkyl; or $R_6$ and $R_7$ together form a ring; are ligands at the central nicotine acetylcholine receptors (nAChRs). The compounds are useful in the treatment of cognitive, neurological or mental disorders in which nAChR dysfunction is involved.




(I)

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| **AT** | Austria | **GB** | United Kingdom | **MR** | Mauritania |
| **AU** | Australia | **GE** | Georgia | **MW** | Malawi |
| **BB** | Barbados | **GN** | Guinea | **NE** | Niger |
| **BE** | Belgium | **GR** | Greece | **NL** | Netherlands |
| **BF** | Burkina Faso | **HU** | Hungary | **NO** | Norway |
| **BG** | Bulgaria | **IE** | Ireland | **NZ** | New Zealand |
| **BJ** | Benin | **IT** | Italy | **PL** | Poland |
| **BR** | Brazil | **JP** | Japan | **PT** | Portugal |
| **BY** | Belarus | **KE** | Kenya | **RO** | Romania |
| **CA** | Canada | **KG** | Kyrgystan | **RU** | Russian Federation |
| **CF** | Central African Republic | **KP** | Democratic People's Republic of Korea | **SD** | Sudan |
| **CG** | Congo | | | **SE** | Sweden |
| **CH** | Switzerland | **KR** | Republic of Korea | **SI** | Slovenia |
| **CI** | Côte d'Ivoire | **KZ** | Kazakhstan | **SK** | Slovakia |
| **CM** | Cameroon | **LI** | Liechtenstein | **SN** | Senegal |
| **CN** | China | **LK** | Sri Lanka | **TD** | Chad |
| **CS** | Czechoslovakia | **LU** | Luxembourg | **TG** | Togo |
| **CZ** | Czech Republic | **LV** | Latvia | **TJ** | Tajikistan |
| **DE** | Germany | **MC** | Monaco | **TT** | Trinidad and Tobago |
| **DK** | Denmark | **MD** | Republic of Moldova | **UA** | Ukraine |
| **ES** | Spain | **MG** | Madagascar | **US** | United States of America |
| **FI** | Finland | **ML** | Mali | **UZ** | Uzbekistan |
| **FR** | France | **MN** | Mongolia | **VN** | Viet Nam |
| **GA** | Gabon | | | | |

## Carbamoyloxy Amine Compounds

The present invention relates to a novel class of carbamoyloxypropylamine or carbamoyloxyethylamine derivatives in which the alkyl backbone contains substituents or is partly incorporated into ring structures. The novel compounds are ligands at the central nicotine acetylcholine receptors (nAChRs) and accordingly useful in the treatment of certain cognitive, neurological and mental disorders.

## Background of the invention

There is a rapidly growing interest in nAChRs in the central nervous system (CNS) as pharmacological and therapeutic targets (Williams et al., *Drug News & Perspectives* **1994**,*7*, 205-223.). There is convincing evidence of major impairments of central acetylcholine (ACh) neurotransmission in patients with Alzheimer's disease (Pomera et al., *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* **1986**,*10*, 553-569.; Perry, *Br. J. Psychiat* **1988**, *152*, 737-740.). Cortical nAChRs are markedly reduced in brain tissues from Alzheimer patients (Giacobini, *J. Neurosci Res.* **1990**, *27*, 548-560; Whitehouse et al., *Brain Res.* **1986**, *371*, 146-151), reflecting the cholinergic deficit associated with Alzheimer's disease. The parietotemporal cortex is the brain area which is most consistently implicated in the cognitive deficits in Alzheimer patients (Gitelman and Prohovnik,*Neurobiol. Aging.*, **1992** *13*, 313-318). Preclinical and clinical data are consistent with the view that nACh receptor agonists are useful in the treatment of Alzheimer's disease (Sahakian et al., *Br.J. Psych.* **1989**, *154*, 797-800; Levin, *Psychopharmacology* **1992** , *108*, 417-431).

In addition to its palliative effects on cognitive deficits in animal models and patients, nicotine, the most extensively studied nAChR ligand, also shows neuroprotective effects in models relevant to Parkinson's disease (Owman et al., *Prog. Brain Res.* **1989**, 79, 267-276; Williams et al., supra). Such observations make nACh receptors potential targets for therapeutic intervention in this neurologic disease (Aubert et al., *J. Neurochem.* **1992**, 58, 529-541). Similarly, nAChR ligands have therapeutic potential in schizophrenia (Adl r t al.,*Biol. Psychiat.* **1992**, 32, 607-616). Furthermore, nicotine and other nAChR agonists have shown effects in animal models of anxiety (Brioni et al., *Eur.J. Pharmacol.* **1993**, *238*, 1-8) and pain

(Qian et al., *Eur. J. Pharmacol.* **1993**, *250*, 13-14; Badio and Daly, *Mol. Pharmacol.* **1994**, *45*, 563-569).

Tobacco smoke contains a variety of substances, but it is beyond doubt that th addictive nature of smoking is attributable to the content of nicotine. Consequently, nAChR ligands may be useful drugs in therapies for smoking cessation (for references, see Williams et al., supra).

Nicotine is the classical nACh receptor agonist, but the number of nACh receptor agonists and antagonists, synthesized or isolated from natural sources, is rapidly growing (Williams et al., supra).

A number of a analogues of carbamylcholine, including N-methylcarbamylcholine (MCC), (Boksa et al., *Eur. J. Pharmacol.* **1989**, *173*, 93-108; Abood et al.,*Pharmacol. Biochem. Behav.* **1988**, *30*, 403-408) and N,N-dimethylcarbamylcholine (DMCC) (Punzi et al., *Biochem. Pharmacol.* **1991**, *41*, 465-467; Sarawati et al.,*Drug Dev. Res.* **1994**, *31*, 142-146) have been synthesized and characterized as nAChR ligands. The compounds synthesized included some N-alkyl-, N,N-dialkyl and cycloalkyl carbamate estes of dimethylethanolamine and the N-methyl carbamate ester of dimethylpropanolamine.

Similar compounds are known from Chemical Abstracts **1961**, *55*, No CA p 6375a which discloses a few *N*-(dimethylcarbamoyloxyalkyl)dialkylamines, i.e. 3-dimethylcarbamoyloxy-*N,N*-dimethylpropylamine, 3-dimethylcarbamoyloxy-*N,N*-diethylpropylamine, 2-dimethylcarbamoyloxy-*N,N*-diethylpropylamine, 1-[3-(dimethylcarbamoyloxy)propyl]piperidine and 2-dimethylcarbamoyloxy-2-phenyl-1-methyl-*N,N*-dimethylethylamine. These compounds are claimed to exhibit choline esterase activity.

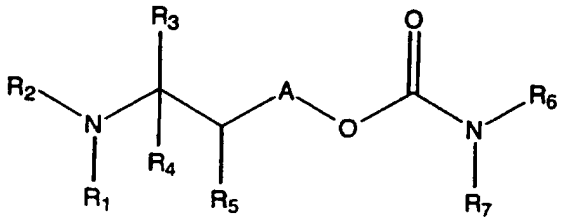
MCC appears to interact with presynaptically localized nAChRs involved in a positive feedback of ACh release (Araujo et al., supra; Lapchak et al., *J. Neurochem.* **1989**, *53*, 1843-1851). Within the compounds found to be nAChR ligands, the quaternary analogues, i.e. carbamate esters of choline, showed markedly higher nAChR affinity than th corr sponding tertiary amines, i.e. carbamate esters of N,N-

dimethylaminoethanol (Abood t al., supra; Punzi et al., supra). Since the former group of analogues are likely to show a limited ability to penetrate the blood-brain barrier it is desired to obtain novel potent nAChR ligands having a good ability to penetrate the blood-brain barrier.

## Summary of the invention

It has now been found that a series of novel tertiary amine homologues of DMCC are potent nAChR ligands.

Accordingly the present invention relates to a novel class of carbamoyloxy amine compounds of the formula I



FORMULA I

wherein A represents $CH_2$ or a bond,

$R_1$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl or phenyl; and $R_2$ is alkyl, alkenyl, alkynyl, cycloalkyl or phenyl; or $R_1$ and $R_2$ together with the adjacent nitrogen form a 3 to 7 membered monoazacyclic ring;

$R_3$ and $R_4$ are the same or different and each represent hydrogen, alkyl, alkenyl, alkynyl, mono- or polyhalogenated lower alkyl, cycloalkyl, phenyl, or phenyl-lower alkyl or $R_3$ and $R_4$ together form a spirojoined $C_{4-7}$ carbocycle; or when $R_1$ and $R_2$ are not linked, $R_3$ and $R_2$ may together with the nitrogen and carbon to which they are attached form a 3 to 7 membered monoazacyclic ring;

$R_5$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, phenyl, or phenyl-lower alkyl; or if $R_2$ do not form a ring with $R_1$ or $R_3$, then $R_5$ and $R_2$ may together with the nitrogen atom to which $R_2$ is attached, the carbon atom substituted with $R_3$ and $R_4$ and the carbon atom to which $R_5$ is attached, form a 3 to 7 membered monoazacyclic ring; or if $R_3$ is not included in a ring and $R_5$ do not form a ring together with $R_2$, $R_5$ and $R_4$ may together with the carbon atoms to which th y ar attached form a 3 to 7 membered carbocyclic ring; provided that $R_5$ is hydrogen when A is a bond;

$R_6$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, phenyl or phenyl-low r alkyl; and $R_7$ is alkyl, alkenyl, alkynyl, cycloalkyl, phenyl or phenyl-lower alkyl, provided that $R_7$ cannot be phenyl or phenyl-lower alkyl when $R_6$ is hydrogen; or

$R_6$ and $R_7$ together with the adjacent nitrogen form a 5 to 6 membered monoazacyclic ring;

with the proviso that one of $R_3$ and $R_4$ must be different from hydrogen when A is a bond, $R_1$ is hydrogen or alkyl and $R_2$ is alkyl, and that $R_3$ and $R_4$ may not both be hydrogen when A represents $CH_2$, $R_6$ is hydrogen or methyl, $R_7$ is methyl and $R_1$ and $R_2$ are both alkyl or together with the N-atom to which they are attached form a piperidine ring;

and pharmaceutically acceptable salts thereof.

The compounds of the invention have been found to have a high affinity for nAChR's. Furthermore, some of the compounds have been found to exhibit nAChR agonist properties. Accordingly, the compounds of the invention are considered useful in the treatment of cognitive, neurological and mental disorders in which nAChR dysfunction is involved, such as pain, dementia, Alzheimers disease, Parkinsons disease, impaired learning ability, impaired memory function, psychosis, schizophrenia, pain and anxiety, in particular dementia, Alzheimers disease or impaired learning ability or memory function. Furthermore, they may be used in theraputical treatment for smoking cessation.

In another aspect the invention provides a pharmaceutical composition comprising at least one novel carbamoyloxy amine compound of formula I in a therapeutically effective amount.

In a further aspect the present invention provides the use of a carbamoyloxy amine compound of formula I for the manufacture of a pharmaceutical preparation for the treatment of the above mentioned disorders and diseases.

**D tailed D scription of th Invention**

Some of the compounds of general Formula I may exist as optical isomers thereof and such optical isomers as well as any mixture thereof, including the racemic

mixtures, ar also embraced by the invention.

In the present context, the term alkyl designates $C_{1-8}$ alkyl which may be straight or branched such as methyl, ethyl, propyl, isopropyl, butyl, tert.butyl, pentyl, hexyl, heptyl or octyl. Among the alkyl groups, lower alkyl groups are preferred. The term lower alkyl designates $C_{1-4}$ alkyl which may be a straight or branched such as methyl, ethyl, propyl, isopropyl, butyl, tert.butyl. Similarly, alkenyl and alkynyl, designate $C_{2-8}$ groups having at least one double or tripple bond, respectively, and lower alkenyl and lower alkynyl designate such groups having up to 4 carbon atoms.

The term cycloalkyl designates a saturated carbocyclic ring having 3-7 carbon atoms, inclusive.

The term phenyl-lower alkyl designates a lower alkyl group (as herein defined) which, in turn, is substituted with a phenyl group. Preferred phenyl-lower alkyl are benzyl, 1-and 2-phenylethyl, 1-,2-, and 3-phenylpropyl, and 1-methyl-1-phenylethyl.

The term halogen designates F, Cl, Br or I, F being preferred. The term polyhalogenated lower alkyl designates lower alkyl, as defined above, substituted with two or more halogen atoms, which may be the same or different. A preferred example of polyhalogenated lower alkyl is trifluoromethyl.

The term a 3 to 7 membered monoazacyclic ring refers to a 3-, 4-, 5-, 6- or 7-membered ring containing one nitrogen atom, such as aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl or perhydroazepinyl. Preferred groups are pyrrolidinyl and piperidinyl.

The term 3 to 7 membered carbocyclic ring refers to a 3-, 4-, 5-, 6- or 7-membered carbon ring, for instance cyclopropane, cyclobutane, cyclopentane, cyclohexane or cycloheptane.

The salts of the compounds of the formula I include any pharmaceutically acceptable acid addition salt. This term as used herein generally includes the non-toxic acid addition salts of compounds of the formula I, formed with non-toxic inorganic or organic acids. For example, the salts include salts with non-toxic inorganic acids,

such as hydrochloric, hydrobromic, sulphuric, sulphamic, nitric, phosphoric and the like; and the salts with organic acids such as acetic, propionic, succinic, fumaric, maleic, tartaric, citric, glycolic, stearic, lactic, malic, pamoic, ascorbic, phenylacetic, glutamic, benzoic, salicylic, sulphonic, sulphanilic, and the like.

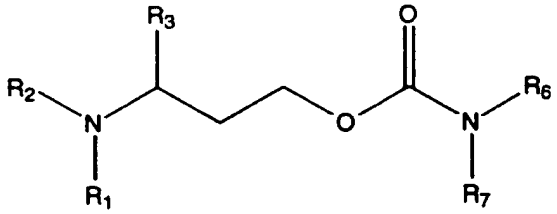
In formula I $R_1$ is preferably lower alkyl, most preferably methyl and $R_2$ is preferably lower alkyl, especially methyl, or designates together with $R_3$ and the nitrogen and carbon, repectively, to which $R_2$ and $R_3$ are attached a pyrrolidinyl ring or $R_1$ and $R_2$ may together form a pyrrolidinyl ring.

Furthermore, $R_3$ is preferably lower alkyl, or together with $R_2$ forms a ring, cf above. Most preferably $R_3$ is lower alkyl, especially methyl.

$R_4$ is preferably hydrogen and $R_5$ is preferably hydrogen or together with $R_3$ forms a ring, cf. above.

$R_6$ is preferably hydrogen or lower alkyl, most preferably hydrogen, ethyl or methyl and $R_7$ is preferably lower alkyl, most preferably ethyl or methyl.

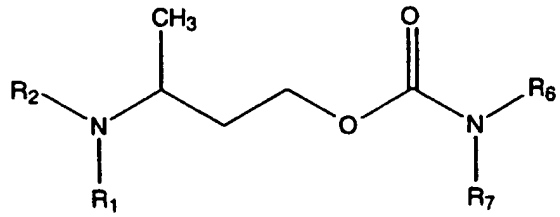
Preferred compounds of the invention are compounds of formula II



FORMULA II

wherein $R_1$, $R_2$, $R_3$, $R_6$ and $R_7$ are as defined above;

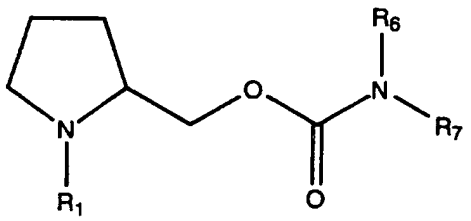
Especially preferred compounds of the invention are compounds of formula III



FORMULA III

wherein $R_1$, $R_2$, $R_6$ and $R_7$ are as defined above;

Another, subclass of preferred compounds of the invention are compounds of formula IV:



Formula IV

wherein $R_1$, $R_6$ and $R_7$ are as defined above.

Especially preferred compounds are:
(R,S)-4-Dimethylcarbamoyloxy-2-*N*,*N*-dimethylbutylamine
(S)-4-Dimethylcarbamoyloxy-2-*N*,*N*-dimethylbutylamine
(R)-4-Dimethylcarbamoyloxy-2-*N*,*N*-dimethylbutylamine

Compounds which may also be mentioned are:
4-Dipropylcarbamoyloxy-2-*N*,*N*-dimethylbutylamine
4-Ethylmethylcarbamoyloxy-2-*N*,*N*-dimethylbutylamine
4-Methylpropylcarbamoyloxy-2-*N*,*N*-dimethylbutylamine

The compounds of the invention are conveniently administered to a patient, via rectal, oral, parenteral or transdermal dosage forms or by inhalation as one or more daily doses, or other time-presented doses. The dose will, of course, depend on the requirements of the individual under treatment.

The effective daily dose of a typical compound is 5.0 µg to 1.5 mg, preferably 10 µg to 1.0 mg, in particular 25 µg to 0.5 mg pr. kg of body weight. Thus, the daily dose is generally in the range of 0.3 to 100 mg, preferably 0.6 to 60mg, usually 1.5 to 40 mg for typical compounds regardless of administration form. The daily dose may be administered in 1 to 3 single doses.

The compounds of the formula I may be administered in the form of tablets,

capsules, suspensions, emulsions, solutions, injectables, suppositories, enema, various drug delivery devices and in other suitable form. The route of administration may be rectally, orally, parenterally or transdermally or by inhalation. The formulation and preparation of any of these dosage forms is well-known to those skilled in the art of pharmaceutical formulation.

In a typical preparation for oral administration e.g. tablet or capsule, any one of the compounds of the present invention in a pharmacologically effective amount is combined with any oral nontoxic pharmaceutically acceptable inert carrier such as lactose, starch and microcrystalline cellulose. Additionally, when required, suitable binders (e.g. gelatin), lubricants (e.g. talc or magnesium stearate) and disintegrating agents (e.g. starch or various cellulose derivatives) are included.

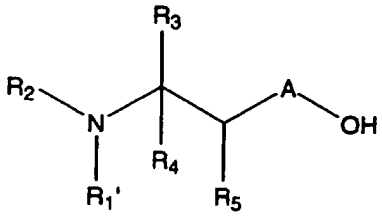
Similarly, in a typical formulation for parenteral application (intravenous, intramuscular, subcutaneous or the like), a compound of the present invention is dissolved in sterile water in a given concentration and sterilized by e.g. membrane filtration, or radiation. The pH of the solution may, if necessary, be adjusted with e.g. hydrochloric acid, sodium hydroxide or a suitable buffer, and a suitable preservative may optionally be added. Similarly, agents like sodium chloride may be added in order to adjust the tonicity of the solution. A suitable parenteral preparation may also consist of the compound formulated as a sterile, solid substance distributed in injection vials. Before dispensing, water for injection is added to dissolve the compound.

For the rectal application of the compounds of the invention, typical dosage forms include suppositories (emulsion and suspension types), rectal gelatin capsules (solutions and suspensions), and enemas or micro-enemas (solutions and suspensions). Thus, in a typical suppository formulation, a compound of the invention is combined with any pharmaceutically acceptable suppository base such as cocoa butter, esterified fatty acids ($C_{10}$-$C_{18}$), glycerinated gelatin, and various water-soluble or dispersible bases like polyethylene glycols and polyoxyethylene sorbitan fatty acid esters.Various additives like salicylates or surfactant mat rials may be incorporated. Enemas or micro-enemas of the solution type may simply be prepared by dissolving compounds of this invention in water or in water containing e.g. 0.5% of methylcellulose or another viscosity-increasing agent.

The novel and useful compounds of the invention may also be administered by drug delivery systems such as gastrointestinal drug delivery devices and rectally applied osmotic delivery devices, wherein the delivery device is manufactured from naturally occurring or synthetic polymeric materials.

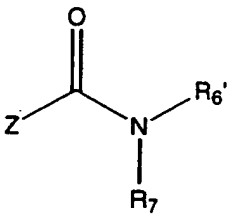
The compounds of the present invention may be prepared by :

a) Reacting a compound of the formula V



Formula V

wherein $R_1$' is as $R_1$ defined above or an amino protecting group, and $R_2$, $R_3$, $R_4$, $R_5$ and A are as defined above, with a compound of the formula VI

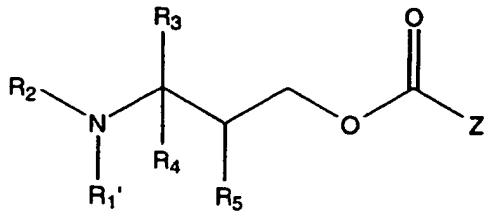


Formula VI

wherein $R_6$' is as defined above for $R_6$ except that it may not be hydrogen, $R_7$ is as defined above, and Z is a leaving group; and then, if an amino protecting group has been used, removing the said group;

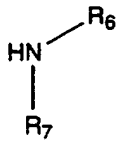
b) reacting a compound of the formula V as defined above with an isocyanate, $R_7$–N=C=O, wherein $R_7$ is as defined above and then, if an amino protecting group has been used; or

c) reacting a compound of the formula VII



Formula VII

wherein $R_1'$, $R_2$, $R_3$, $R_4$, $R_5$, Z and n are as defined above, with a compound of the formula VIII



Formula VIII

wherein $R_6$ and $R_7$ are as defined above and then, if an amino protecting group has been used, removing the said group.

The reaction is carried out without a solvent or in a solvent, e.g. toluene, tetrahydrofuran, diethylether, acetonitrile, N,N-dimethylformamide (DMF), dimethyl sulfoxide, or the like. In a) and c) a base, e.g. sodium hydride, tertiary amine, pyridine, potassium carbonate, or the like, usually has to be present whereas in b) a base may be present if convenient. In a) and b) the temperature during the reaction is usually between 0°C and the boiling point of the mixture and in c) it is usually between -10°C and the boiling point of the mixture The reaction time is normally from 1 to 96 hours.

The compounds formulas V, VI and VIII are either commercially available or may be prepared by standard methods.

One way to obtain the compounds of formula VII may be by reacting a compound of formula V with phosgene.

Suitable leaving groups Z may easily be selected by a person skilled in the art. As examples may be mentioned chlorine, bromine and iodine.

The term amino protecting group designates groups readily removable by hydrolysis or hydrogenation. Suitable protecting groups may easily be selected. As examples of such groups may be mentioned methyloxycarbonyl, ethyloxycarbonyl, tert.butyloxycarbonyl, benzyloxycarbonyl, formyl, acetyl, trityl, benzyl, or the like.

The present invention is further illustrated by the following examples which, however, may not be construed to be limiting.

**Example 1**

(S)-2-Dimethylcarbamoyloxymethyl-1-methylpyrrolidine (Comp. (S)-**1**). Salt with 1.0 equivalent of tartaric acid

To a suspension of 80% sodium hydride dispersion (3.52 g; 117.4 mmol) in dry toluene (70 ml) was added a solution of (S)-2-hydroxymethyl-1-methylpyrrolidine (10.00 g; 87.0 mmol ) in dry toluene (30 ml) over a period of 15 min. After 5 hours at 25°C, a solution of dimethylcarbamoyl chloride (11.20 g;104.4 mmol) in dry toluene (30 ml) was added over a period of 45 min., and the mixture was allowed to stir overnight at 25°C. Water (75 ml) was added, and the two phases were separated. The water phase was extracted with toluene (4 X 75 ml), and the combined organic phases dried with $MgSO_4$. The solvent was removed under reduced pressure and the crude product (15.8 g) was purified by flash column chromatography on silica gel by use of triethylamine:toluene (1:20) and an increasing amount of ethyl acetate as eluent. The eluent was removed under reduced pressure and the purified product was obtained as a yellow oil (15.8 g; 98%). Part of the oil (1.40 g ) was dissolved in dry isopropanol (5 ml) and a solution of L(+)-tartaric acid (1.24 g) in isopropanol (20 ml) was added followed by diethylether. After standing 72 hours at 5°C the title compound was isolated by filtration. Recrystallization from isopropanol and diethyl-ether gave the title compound (1.95 g; 77%), mp.: 82-84°C.

**Example 2**

(S)-2-Ethylcarbamoyloxymethyl-1-methylpyrrolidine (Comp. (S)-**2**). Salt with 1.0 equivalent of fumaric acid.

Ethyl isocyanate (1.23 ml; 15.7 mmol) was added to (S)-2-hydroxymethyl-1-methyl-pyrrolidine (1.50 g; 13.0 mmol) in dry toluene (10 ml) over a period of 5 min.. After 6 hours at 25°C another portion of ethylisocyanate (0.30 ml; 3.93 mmol ) was added. The reaction mixture was stirred overnight. The solvent was removed under reduced pressure and the crude product (2.22 g; 92%) was purified by flash column chroma-tography on silica gel by use of triethylamine:heptane (1:20) and an increasing amount of ethyl acetate as elu nt. The eluent was evaporated under reduced pressure to give the free base of the title compound (1.96 g; 81%) as an oil. As described in Example 1, part of the oil (0.50 g) was converted to the fumaric acid salt by using fumaric acid. Recrystallization from isopropanol and diethylether gave

th title compound (0.63 g; 77%), mp.: 101.5-102.5°C.

**Example 3**

(R,S)-4-Dimethylcarbamoyloxy-2-*N,N*-dimethylbutylamine (Comp. **8**). Salt with 1.0 equivalent of fumaric acid.

(R,S)-3-*N,N*-Dimethylamino-1-butanol (4.29 g; 36.7 mmol) was added over a period of 10 min to a suspension of 60% sodium hydride dispersion (1.91 g; 47.7 mmol) in dry DMF (100 ml). After 3 hours at 40°C, dimethylcarbamoyl chloride (5.12 g; 47.7 mmol) was added over a period of 20 min. After another 4 hours at 40°C, the mixture was allowed to stir overnight at 25°C. After removal of the solvent under reduced pressure the residue was taken up in 100 ml water and extrated with diethylether (80 ml +3x30 ml). The combined organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure, leaving the crude product as a yellow oil (5.69 g; 83%). As described in Example 1, the oil was converted to the fumaric acid salt by using fumaric acid. Recrystallization from isopropanol and diethylether gave the title compound (5.78 g; 61%), mp.: 91-93.5°C.

**Example 4**

*Cis-N*-Methyl-2-dimethylcarbamoyloxymethylcyclopentylamine (Comp. cis-(1S,2R)-**18**). Salt with 1.0 equivalent fumaric acid.

*Cis,trans*-2-(*N*-benzyl-*N*-methylamino)cyclopentanemethanol (4.50 g; 18.4 mmol ) was reacted with dimethylcarbamoyl chloride (2.67 g; 24.8 mmol ) as described in Example 2. The crude product (5.55 g; 96%) was purified by flash column chromatography on silica gel by use of triethylamine:heptane (1:20) as eluent. The eluent was evaporated under reduced pressure and the *N*-benzylated title compound was obtained as an oil (1.85 g; 32%). Part of the oil (1.55 g; 5.34 mmol) was dissolved in acetic acid (50 ml) and hydrogenated with 120 mg prereduced $PtO_2$ at 3 atm.and 25°C for 24 hours. The solvent was removed under reduced pressure, and the crude product (1.09 g; 99%) was purified by flash column chromatography on silica gel by use of triethylamine:heptane (1:20) as eluent. The eluent was evaporated under reduced pressure to give the free base of the title compound (0.59 g; 54%) as an oil. As described in Example 1, the oil was converted to the fumaric acid salt. Recrystallization from isopropanol and diethylether gave the title compound (0.54 g; 80%), mp.: 102.5-103.5°C.

The following Table 1 lists further examples of the invention. The symbols used in the table refers to formula I. For the purpose of completion the compounds of Examples 1-4 are also included in the table.

The compounds were synthesized by methods analogeous to those described in Examples 1-4. Compounds (S)-8 and (R)-8 were prepared from Comp. 8 by standard resolution methods.

The following optical rotations have been measured, $[\alpha]_D^{25}$ deg.:

Comp. (S)-1: +3.7; Comp. (R)-1: -3.7; Comp. cis-(1S,2R)-18: -52.9; and Comp. cis-(1R,2S)-18: +52.7, Comp. (S)-8 (tartrate): -4.41, Comp. (R)-8 (tartrate): +4.97, (R)-30: +4.81 and (S)-30: 4.98.

Table 1: Examples

| Comp. | A[1)] | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | Mp °C (salt)[2)] |
|---|---|---|---|---|---|---|---|---|---|
| (S)-1 | B | Me | $(CH_2)_3$ | | H | H | Me | Me | 130.5-132.5 (T) |
| (R)-1 | B | Me | $(CH_2)_3$ | | H | H | Me | Me | 130-132 (B) |
| (S)-2 | B | Me | $(CH_2)_3$ | | H | H | H | Et | 101.5-102.5 |
| (S)-3 | B | Me | $(CH_2)_3$ | | H | H | Et | Et | 102-103 |
| (S)-4 | B | Me | $(CH_2)_3$ | | H | H | H | Me | 76-77.5 |
| (S)-5 | B | Et | $(CH_2)_3$ | | H | H | Me | Me | 82-84 |
| 6 | B | Me | $(CH_2)_4$ | | H | H | Me | Me | 123-124.5 |
| 7 | $CH_2$ | Me | $(CH_2)_3$ | | H | H | Me | Me | 95-96 |
| 8 | $CH_2$ | Me | Me | Me | H | H | Me | Me | 91-93.5 |
| (s)-8 | $CH_2$ | Me | Me | Me | H | H | Me | Me | 120 dec. (DT) |
| (R)-8 | $CH_2$ | Me | Me | Me | H | H | Me | Me | 120 dec. (DT) |
| 9 | $CH_2$ | Me | Me | Et | H | H | Me | Me | 102-103 |
| 10 | $CH_2$ | Me | Me | $Me_2CH$ | H | H | Me | Me | 96-100 |
| 11 | $CH_2$ | Me | Me | $C_6H_{11}$ | H | H | Me | Me | 130-133 |

| Comp. | A* | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | Mp °C |
|---|---|---|---|---|---|---|---|---|---|
| 12 | $CH_2$ | Me | Me | $(CH_2)_5$ | | H | Me | Me | 114-115 |
| 13 | $CH_2$ | H | Me | Me | H | H | Me | Me | 66-68 |
| 14 | $CH_2$ | $(CH_2)_4$ | | Me | H | H | Me | Me | 98-99 |
| 15 | $CH_2$ | $(CH_2)_5$ | | Me | H | H | Me | Me | 121-125 |
| 16 | $CH_2$ | Me | Me | H | H | H | H | Et | 84-87 |
| 17 | $CH_2$ | Me | Me | H | H | H | Et | Et | 60-65 |
| cis-18 | $CH_2$ | Me | Me | H | $(CH_2)_3$ | | Me | Me | 126-127 |
| trans-18 | $CH_2$ | Me | Me | H | $(CH_2)_3$ | | Me | Me | 122-124.5 |
| cis-(1S2R)18 | $CH_2$ | Me | Me | H | $(CH_2)_3$ | | Me | Me | 146.5-167.5 (T) |
| cis-(1R,2S)18 | $CH_2$ | Me | Me | H | $(CH_2)_3$ | | Me | Me | 146.5-167.5 (T) |
| 19 | $CH_2$ | H | Me | H | $(CH_2)_3$ | | Me | Me | 102.5-103.5 |
| 20 | $CH_2$ | Me | Me | H | $(CH_2)_4$ | | Me | Me | 146-148 |
| 21 | $CH_2$ | $(CH_2)_4$ | | H | H | H | Me | Me | 103.5-104.5 |
| 22 | B | Me | Me | Me | Me | H | Me | Me | 144.5-145.5 |
| 23 | B | $(CH_2)_4$ | | H | H | H | Me | Me | 110-113 |
| 24 | B | $(CH_2)_4$ | | H | H | H | H | Et | 62-64 (M) |
| 25 | B | $(CH_2)_5$ | | H | H | H | Me | Me | 118-122 |
| 26 | B | $(CH_2)_5$ | | H | H | H | H | Et | 88-93 |
| 27 | B | Me | Me | Me | H | H | Me | Me | 112-117 |
| 28 | $CH_2$ | Me | $R_2$-$R_5$=$(CH_2)_2$ ; | | $R_3$, $R_4$= H | | Me | Me | 84-85 |
| 29 | $CH_2$ | Me | $R_2$-$R_5$=$(CH_2)_3$ ; | | $R_3$, $R_4$= H | | Me | Me | 112-113 |
| 30 | $CH_2$ | Me | Me | Me | H | H | H | Me | 97-99 |
| (R)-30 | $CH_2$ | Me | Me | Me | H | H | H | Me | 91-91 (O) |
| (S)-30 | $CH_2$ | Me | Me | Me | H | H | H | Me | 91-92 (O) |
| 31 | $CH_2$ | Me | Me | Me | H | H | Et | Et | 96-97.5 (C) |

1): B designates a bond.

2): T designates tartrate, B hydrobromide, DT dibenzoyl tartrat ; M maleate, O oxalate and C hydrochloride. The remaining compounds were isolated as fumarates.

**Pharmacology**

The compounds of the invention were tested in the following well recognized and reliable test methods.

**L-[$^3$H]Nicotine binding**

L-[$^3$H]Nicotine binding to cholinergic receptors in rat brain membranes was performed essentially as described by Lippiello, P.M. and Fernandes, K.G. *Mol. Pharmacol,* **1986**, *29*, 448-454.. Rat brains were homogenized (Ultraturrax) in 10 vol (w/v) buffer consisting of $Na_2HPO_4$, 8 mM; $KH_2PO_4$, 1.5 mM; KCl, 3 mM; NaCl, 120 mM; EDTA, 2 mM; HEPES, 20 mM; and iodoacetamide, 5mM (pH 7.4). The homogenate was centrifuged (50.000 x g; 20 min.; 0°C) and the pellet resuspended in 10 vol. cold standard assay buffer with the same composition as the buffer preparation described above, except for the addition of $MgCl_2$, 1mM and $CaCl_2$, 2 mM, and the elimination of EDTA and iodoacetamide. Aliquots (0.1 mg of tissue) were incubated with 5 nM L-[$^3$H]Nicotine (78 Ci/mmol, Amersham) alone or in the presence of test compound in a total volume of 0.6 ml for 60 min. at 0°C. Incubation was terminated by adding 5 ml of ice-cold sodium potassium phosphate buffer (50 mM, pH 7.4) followed by rapid filtration through Whatman GF/B filters presoaked in 0.1%polyethyleneimine using a Brandel cell-harvester. Filters were washed with three 5-ml aliquots of cold sodium potassium phosphate buffer, and bound radioactivity estimated by liquid scintillation counting methods. Each compound was tested in three different concentrations, and nonspecific binding estimated at 0.5 µM nicotine-H-tartrate. All estimations were made in triplicate, and each displacement experiment was repeated at least three times.

Table 2: L-[$^3$H] Nicotine Binding Data

| Compound No | $IC_{50}$-values (μM) L-[$^3$H] Nicotine | Compound No | $IC_{50}$-values (μM) L-[$^3$H] Nicotine |
|---|---|---|---|
| 1 | 0.068 | cis-18 | 0.56 |
| (S)-1 | 0.52 | trans-18 | 2.3 |
| (S)-2 | 0.66 | cis-(1S,2R)-18 | 0.32 |
| (S)-3 | 0.026 | cis-(1R,2S)-18 | 0.65 |
| (S)-4 | 0.30 | 19 | 5.4 |
| (S)-5 | 0.59 | 20 | 27 |
| 6 | 11 | 21 | 1.8 |
| 7 | 1.6 | 22 | 67 |
| 8 | 0.009 | 23 | 0.63 |
| (S)-8 | 0.064 | 24 | 2.2 |
| (R)-8 | 0.003 | 25 | 11 |
| 9 | 0.15 | 26 | 47 |
| 10 | 2.9 | 27 | 2.3 |
| 11 | 80 | 28 | 6.0 |
| 12 | 29 | 29 | 47 |
| 13 | 1.6 | 30 | 0.008 |
| 14 | 33 | (R)-30 | 0.006 |
| 16 | 0.64 | (S)-30 | 0.39 |
| 17 | 0.84 | 31 | 0.014 |

Furthermore, the compounds of the invention have been tested with respect to affinity for muscarinic receptors by the method of Sauerberg, P. et al., *J. Med. Chem.* **1988**, *31*, 1312-1316, and some of compounds were tested with respect to agonistic effect at the nAChRs in the Guinea Pig Ileum test as described by Arnt et al. Eur. J. Pharmacol. **1992**, *218*, 159-169 or the Nicotine Cue test as described by L. T. Meltzer et al., Psychopharmacology 68, 283-286, 1980. Some of the compounds were selective towards the nAChRs as compared to muscarinic receptors whereas the compounds tested in the Guinea Pig Ileum test or the Nicotine Cue test were found to act as agonists.

**Formulation Examples**

The pharmaceutical formulations of the invention may be prepared by conventional methods in the art as described above.

Typical examples of recipes for the formulation of the invention are as follows:

1) Tablets containing 5.0 mg of active compound calculated as the free base:

| | |
|---|---|
| Compound cis-(1S,2R)-18 | 5.0 mg |
| Lactose | 60 mg |
| Maize starch | 30 mg |
| Hydroxypropylcellulose | 2.4 mg |
| Microcrystalline cellulose | 19.2 mg |
| Croscarmellose Sodium Type A | 2.4 mg |
| Magnesium stearate | 0.84 mg |

2) Tablets containing 10 mg of Compound **8** calculated as the free base:

| | |
|---|---|
| Compound **8** | 10 mg |
| Lactose | 46.9 mg |
| Maize starch | 23.5 mg |
| Povidone | 1.8 mg |
| Microcrystalline cellulose | 14.4 mg |
| Croscarmellose Sodium Type A | 1.8 mg |
| Magnesium stearate | 0.63 mg |

3) Syrup containing per millilitre:

| | |
|---|---|
| Compound (S)-**1** | 25 mg |
| Sorbitol | 500 mg |
| Hydroxypropylcellulose | 15 mg |
| Glycerol | 50 mg |
| Methyl-paraben | 1 mg |
| Propyl-paraben | 0.1 mg |
| Ethanol | 0.005 ml |
| Flavour | 0.05 mg |

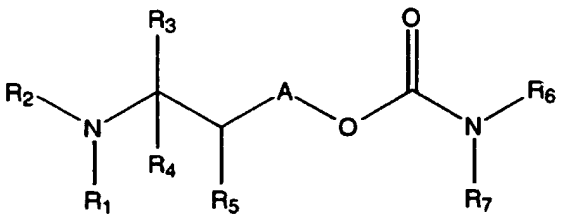
| | |
|---|---|
| Saccharin natrium | 0.5 mg |
| Water | ad 1 ml |

4) Solution for injection containing per millilitre:

| | |
|---|---|
| Compound **8** | 20 mg |
| Sorbitol | 5.1 mg |
| Acetic acid | 0.08 mg |
| Water for injection | ad 1 ml |

## PATENT CLAIMS

1. A carbamoyloxypropylamine or carbamoyloxyethylamine compounds of the formula I



FORMULA I

wherein A represents $CH_2$ or a bond,

$R_1$ is hydrogen, $C_{1-8}$ alkyl, $C_{2-8}$ alkenyl, $C_{2-8}$ alkynyl, $C_{3-7}$ cycloalkyl or phenyl; and $R_2$ is $C_{1-8}$ alkyl, $C_{2-8}$ alkenyl, $C_{2-8}$ alkynyl, $C_{3-7}$ cycloalkyl or phenyl; or $R_1$ and $R_2$ together with the adjacent nitrogen form a 3 to 7 membered monoazacyclic ring;

$R_3$ and $R_4$ are the same or different and each represent hydrogen, $C_{1-8}$ alkyl, $C_{2-8}$ alkenyl, $C_{2-8}$ alkynyl, mono- or polyhalogenated $C_{1-4}$ alkyl, $C_{3-7}$ cycloalkyl, phenyl, or phenyl-$C_{1-4}$ alkyl or $R_3$ and $R_4$ together form a spirojoined $C_{4-7}$ carbocycle; or when $R_1$ and $R_2$ are not linked, $R_3$ and $R_2$ may together with the nitrogen and carbon to which they are attached form a 3 to 7 membered monoazacyclic ring;

$R_5$ is hydrogen, $C_{1-8}$ alkyl, $C_{2-8}$ alkenyl, $C_{2-8}$ alkynyl, $C_{3-7}$ cycloalkyl, phenyl, or phenyl-$C_{1-4}$ alkyl; or

if $R_2$ do not form a ring with $R_1$ or $R_3$, then $R_5$ and $R_2$ may together with the nitrogen atom to which $R_2$ is attached, the carbon atom substituted with $R_3$ and $R_4$ and the carbon atom to which $R_5$ is attached, form a 3 to 7 membered monoazacyclic ring;

or if $R_3$ is not included in a ring and $R_5$ do not form a ring together with $R_2$, $R_5$ and $R_4$ may together with the carbon atoms to which they are attached form a 3 to 7 membered carbocyclic ring; provided that $R_5$ is hydrogen when A is a bond;

$R_6$ is hydrogen, $C_{1-8}$ alkyl, $C_{2-8}$ alkenyl, $C_{2-8}$ alkynyl, $C_{3-7}$ cycloalkyl, phenyl or phenyl-$C_{1-4}$ alkyl; and $R_7$ is $C_{1-8}$ alkyl, $C_{2-8}$ alkenyl, $C_{2-8}$ alkynyl, $C_{3-7}$ cycloalkyl, phenyl or phenyl-$C_{1-4}$ alkyl, provided that $R_7$ cannot be phenyl or phenyl-$C_{1-4}$ alkyl when $R_6$ is hydrogen; or

$R_6$ and $R_7$ together with the adjacent nitrogen form a 5 to 6 membered monoazacyclic ring;

with the proviso that one of $R_3$ and $R_4$ must be different from hydrogen when A is a

bond, $R_1$ is hydrogen or $C_{1-8}$ alkyl and $R_2$ is $C_{1-8}$ alkyl, and that $R_3$ and $R_4$ may not both be hydrogen when A represents $CH_2$, $R_6$ is hydrogen or methyl, $R_7$ is methyl and $R_1$ and $R_2$ are both $C_{1-8}$ alkyl or together with the N-atom to which they are attached form a piperidine ring;

or a pharmaceutically acceptable salt thereof.

**2.** A compound of Claim **1**, **characterized in** that $R_1$ and $R_2$ are both $C_{1-4}$ alkyl, preferably methyl.

**3.** A compound of Claim **1**, **characterized in** that $R_2$ together with $R_3$ and the nitrogen and carbon, repectively, to which $R_2$ and $R_3$ are attached, designates a pyrrolidinyl ring.

**4.** A compound of Claim **1**, **characterized in** that $R_1$ and $R_2$ together with the nitrogen atom to which they are attached form a pyrrolidinyl ring.

**5.** A compound of Claim **1**, **characterized in** that $R_3$ is $C_{1-4}$ alkyl, preferably methyl

**6.** A compound of Claim **1**, **characterized in** that $R_4$ together with $R_5$ and the carbon atoms to which they are attached form a cyclopentyl ring.

**7.** A compound of Claim **1**, **characterized in** that $R_4$ and $R_5$ are hydrogen.

**8.** A compound of any of Claims **1 - 7**, **characterized in** that $R_6$ is hydrogen or $C_{1-4}$ alkyl, preferably hydrogen, ethyl or methyl and $R_7$ is $C_{1-4}$ alkyl, preferably ethyl or methyl.

**9.** A compound of any of Claims **1 - 8**, **characterized in** that A is $CH_2$ and $R_4$ and $R_5$ are hydrog n.

**10.** A compound of Claims **9**, **charact rized in** that $R_3$ is methyl.

**11.** A compound of Claim **1**, **characterized in** that $R_2$ together with $R_3$ and the nitrogen and carbon, repectively, to which $R_2$ and $R_3$ are attached, designates a pyrrolidinyl ring, A represents a bond and $R_4$ and $R_5$ are hydrogen.

**12.** A compound of Claim **1**, **characterized in** that it is selected from the group consisting of:
(S)-2-Dimethylcarbamoyloxymethyl-1-methylpyrrolidine,
4-Dimethylcarbamoyloxy-2-*N,N*-dimethylbutylamine,
(R,S)-4-Dimethylcarbamoyloxy-2-*N,N*-dimethylbutylamine,
(S)-4-Dimethylcarbamoyloxy-2-*N,N*-dimethylbutylamine,
(R)-4-Dimethylcarbamoyloxy-2-*N,N*-dimethylbutylamine and
*Cis*-*N*-Methyl-2-dimethylcarbamoyloxymethylcyclopentylamine; and
pharmaceutically acceptable salts thereof.

**13.** A pharmaceutical composition, **characterized in** that it comprises at least one compound of any of Claims **1** - **12**, in a therapeutically effective amount, together with one or more pharmaceutically acceptable carriers or diluents.

**14.** Use of a compound of any of Claims **1** - **12** for the manufacture of a pharmaceutical preparation for the treatment of a cognitive, neurological or mental disorders in which nAChR dysfunction is involved, preferably pain, dementia, Alzheimers disease, Parkinsons disease, impaired learning ability, impaired memory function, psychosis, schizophrenia, pain or anxiety or for theraputical treatment for smoking cessation.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00368

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07C 271/12, C07D 207/08, C07D 211/22, C07D 295/088, A61K 31/27, A61K 31/40, A61K 31/495

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | FR 2704M A (SIEGFRIED, SOCIETE ANONYME RESIDANT EN SUISSE), 3 August 1964 (03.08.64) -- | 1-2,4-5, 7-10,13-14 |
| X | CH 405266 A (SIEGFRIED AKTIENGESELLSCHAFT), 15 July 1966 (15.07.66) -- | 1-2,13-14 |
| X | US 2794810 A (JOHN W. CUSIC), 4 June 1957 (04.06.57) -- | 1-2,4-5, 7-10,13 |
| X | CH 467755 A (SIEGFRIED AKTIENGESELLSCHAFT), 14 March 1969 (14.03.69) -- | 1-2,4-5, 7-10,13 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 22 December 1995 | 02 -01- 1996 |
| Name and mailing address of the ISA/<br>Swedish Patent Offic<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Göran Karlsson<br>Telephone No. +46 8 782 25 00 |

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00368

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

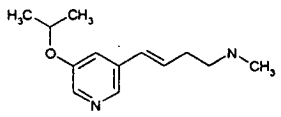
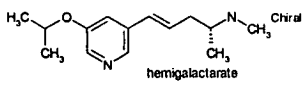
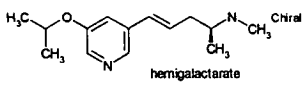
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | Chemical Abstracts, Volume 55, 25 December 1961 (25.12.61), (Columbus, Ohio, USA), THE ABSTRACT No 6375a, JP, 7464, A, (Dainippon Pharmaceutical Co., Ltd) 20 June 1961 (20.06.61) -- | 1-2,4-5, 7-10,13 |
| X | CH 468978 A (SIEGFRIED AKTIENGESELLSCHAFT), 15 April 1969 (15.04.69) -- | 1-2,5,7,13 |
| X | US 3347856 A (CARL D. LUNSFORD), 17 October 1967 (17.10.67) -- | 1,13-14 |
| X | US 3287471 A (JOHN W. CUSIC ET AL), 22 November 1966 (22.11.66) -- | 1 |

## INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/DK 95/00368

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| FR-A- 929707 | 03/08/64 | NONE | | |
| CH-A- 405266 | 15/07/66 | NONE | | |
| US-A- 2794810 | 04/06/57 | NONE | | |
| CH-A- 467755 | 14/03/69 | DE-A- | 1470277 | 29/05/69 |
| JP-A- 7464 | 20/06/61 | NONE | | |
| CH-A- 468978 | 15/04/69 | DE-A- | 1470277 | 29/05/69 |
| US-A- 3347856 | 17/10/67 | BE-A- | 676170 | 08/08/66 |
| | | CH-A- | 467798 | 00/00/00 |
| | | FR-A- | 1467524 | 00/00/00 |
| | | GB-A- | 1136104 | 00/00/00 |
| | | LU-A- | 50404 | 08/08/66 |
| | | NL-A- | 6601380 | 09/08/66 |
| | | OA-A- | 1912 | 04/02/70 |
| | | BE-A- | 676256 | 16/06/66 |
| | | CH-A- | 466282 | 00/00/00 |
| | | DE-A- | 1620184 | 12/03/70 |
| | | FR-A- | 1490497 | 00/00/00 |
| | | GB-A- | 1128351 | 00/00/00 |
| | | NL-A- | 6600234 | 10/08/66 |
| US-A- 3287471 | 22/11/66 | NONE | | |

# Table 5

| Compound | STRUCTURE | Ki | α4β2 Emax | α4β2 EC50 | Activity Ratio Emax/EC50 | Cp max (ng/mL) | AUC 0-∞ (h.ng/mL) |
|---|---|---|---|---|---|---|---|
| 1 |  | 5 | 59 | 379 | 0.15 | 19 | 30 |
| 2 |  hemigalactarate | 62 | 14 | 88 | 0.16 | 28 | 50 |
| 3 |  hemigalactarate | 11 | 57 | 220 | 0.26 | 39 | 123 |